Division of Nephrology
Department of Medicine
Helsinki University Central Hospital

Folkhälsan Institute of Genetics
Folkhälsan Research Center

Research Programs Unit
Diabetes and Obesity
University of Helsinki
Helsinki, Finland

Lipid profile and micro- and macrovascular complications in type 1 diabetes

Nina Tolonen

ACADEMIC DISSERTATION

To be presented,
with the permission of the Medical Faculty of the University of Helsinki,
for public examination in Auditorium 2, Biomedicum Helsinki,
on January 23rd 2015, at 12 noon.

Helsinki 2015

Supervised by **Professor Per-Henrik Groop**
Division of Nephrology, Department of Medicine
Helsinki University Central Hospital, Finland

Folkhälsan Institute of Genetics
Folkhälsan Research Center, Helsinki, Finland

Research Programs Unit, Diabetes and Obesity
University of Helsinki, Finland

Reviewed by **Docent Jorma Lahtela**
School of Medicine
University of Tampere, Finland

Department of Internal Medicine
Tampere University Hospital, Finland

and

Professor Timo Strandberg
University of Helsinki, and
Helsinki University Central Hospital
Geriatrics, Helsinki, Finland

University of Oulu
Institute of Health Sciences/Geriatrics, and
Unit of General Practice
Oulu University Hospital, Oulu, Finland

Opponent **Emeritus Professor John Fuller**
Department of Epidemiology and Public Health
University College London, United Kingdom

ISBN 978-951-51-0567-7 (paperback)
ISBN 978-951-51-0568-4 (PDF)
http://ethesis.helsinki.fi
Unigrafia
Helsinki 2015

To my family, friends, colleagues, and all FinnDiane patients

He who studies medicine without books
sails an uncharted sea,
but he who studies medicine without patients
does not go to sea at all.

Sir William Osler

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Tolonen N, Forsblom C, Thorn L, Wadén J, Rosengård-Bärlund M, Saraheimo M, Heikkilä O, Pettersson-Fernholm K, Taskinen M-R, Groop P-H, FinnDiane Study Group. Relationship between lipid profiles and kidney function in patients with type 1 diabetes. *Diabetologia* 51:12–20, 2008

II Tolonen N, Forsblom C, Thorn L, Wadén J, Rosengård-Bärlund M, Saraheimo M, Feodoroff M, Mäkinen VP, Gordin D, Taskinen M-R, Groop P-H, FinnDiane Study Group. Lipid abnormalities predict progression of renal disease in patients with type 1 diabetes. *Diabetologia* 52:2522-2530, 2009

III *Tolonen N, *Hietala K, Forsblom C, Harjutsalo V, Mäkinen V-P, Kytö J, Summanen PA, Thorn LM, Wadén J, Gordin D, Taskinen M-R, Groop P-H, FinnDiane Study Group. Associations and interactions between lipid profiles, retinopathy and nephropathy in patients with type 1 diabetes: the FinnDiane Study. *Journal of Internal Medicine* 274:469-479, 2013

IV Tolonen N, Forsblom C, Mäkinen V-P, Harjutsalo V, Gordin D, Feodoroff M, Sandholm N, Thorn LM, Wadén J, Taskinen M-R, Groop P-H, FinnDiane Study Group. Different lipid variables predict incident coronary artery disease in patients with type 1 diabetes with or without diabetic nephropathy: the FinnDiane Study. *Diabetes Care* 37:2374-2382, 2014

*equal contribution

These publications are referred to in the text by their Roman numerals and have been reprinted with permission from their copyright holders.

ABBREVATIONS

ACCORD	Action to Control Cardiovascular Risk in Diabetes
ACE	Angiotensin-converting enzyme
ACR	Albumin-to-creatinine ratio
AdDIT	Adolescent type 1 Diabetes, cardio-renal Intervention Trial
ADVANCE	Action in Diabetes and Vascular Disease
AER	Albumin excretion rate in urine
AGE	Advanced glycation end-product
ALERT	Assessment of Lescol in Renal Transplantation
ALLHAT-LLT	Lipid-Lowering Treatment to Prevent Heart Attack Trial - Lipid-Lowering Trial
Apo	Apolipoprotein
ARB	Angiotensin II receptor blocker
ARIC	Atherosclerosis Risk in Communities
ASCOT-LLA	Anglo-Scandinavian Cardiac Outcomes Trial - Lipid-Lowering Arm
AUC	Area under the curve
BMI	Body mass index
CAD	Coronary artery disease
CAN	Cardiovascular autonomic neuropathy
CARDS	Collaborative Atorvastatin Diabetes Study
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRP	C-reactive protein
CVD	Cardiovascular disease
DAIS	Diabetes Atherosclerosis Intervention Study
DBP	Diastolic blood pressure
DCCT	Diabetes Control and Complications Trial
DCCT/EDIC	Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study
DPN	Distal symmetric polyneuropathy
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
FinnDiane	Finnish Diabetic Nephropathy Study
eGDR	Estimated glucose disposal rate
eGFR	Estimated glomerular filtration rate
ERFC	Emerging Risk Factor Collaboration
ESRD	End-stage renal disease
ETDRS	Early Treatment of Diabetic Retinopathy Study
GADA	Glutamic acid decarboxylase antibodies
GENIE	Genetics of Nephropathy - an International Effort
GFR	Glomerular filtration rate
HbA_{1c}	Glycosylated hemoglobin A_{1c}
HDL	High-density lipoprotein
HL	Hepatic lipase

HLA	Human leukocyte antigen
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
HPS	Heart Protection Study
HR	Hazard ratio
ICD	International Classification of Diseases
IDL	Intermediate-density lipoprotein
IL	Interleukin
IQR	Interquartile range
JUPITER	Justification for the Use of Statins in Prevention - an Intervention Trial Evaluating Rosuvastatin
LADA	Latent Autoimmune Diabetes of the Adult
LCAT	Lecithin–cholesterol acyltransferase
LDL	Low-density lipoprotein
Ln	Natural logarithm
Lp(a)	Lipoprotein(a)
LPL	Lipoprotein lipase
MDRD	Modification of Diet in Renal Disease
MODY	Maturity onset diabetes of the young
NNT	Number needed to treat
NADPH	Nicotinamide adenine dinucleotide phosphate
NPDR	Non-proliferative diabetic retinopathy
NRI	Net reclassification improvement
OR	Odds ratio
oxLDL	Oxidized LDL
PDR	Proliferative diabetic retinopathy
Pittsburgh EDC	Pittsburgh Epidemiology of Diabetes Complications
PLTP	Phospholipid transfer protein
ROC	Receiver-operating characteristic
ROS	Reactive oxygen species
RR	Relative risk
SBP	Systolic blood pressure
SD	Standard deviation
sdLDL	Small, dense LDL
Swedish NDR	Swedish National Diabetes Register
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
TNT	Treating to New Targets
TRL	Triglyceride-rich lipoprotein
UKPDS	United Kingdom Prospective Diabetes Study
VADT	Veterans Affairs Diabetes Trial
VLDL	Very-low-density lipoprotein
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy
WHO-MSVDD	World Health Organization Multinational Study of Vascular Disease in Diabetes
WHR	Waist-to-hip ratio

ABSTRACT

Background
Cardiovascular disease is the most common cause of death in patients with type 1 diabetes (1), and the premature mortality rates are especially high in patients with diabetic nephropathy (2, 3). Diabetic retinopathy is the leading cause of vision loss among the working-age population in industrialized countries (4). Early identification and aggressive treatment of risk factors are crucial to reduce the incidence of diabetic complications.

Aims
To examine the relationships between lipid profiles and diabetic nephropathy, diabetic retinopathy, and incident coronary artery disease (CAD) events in a large nationwide cohort of patients with type 1 diabetes.

Subjects and methods
These studies are part of the ongoing Finnish Diabetic Nephropathy Study (FinnDiane), a nationwide, multicenter study aimed at identifying both genetic and clinical risk factors for the development of diabetic complications in patients with type 1 diabetes. Studies I (N=2927) and III (N=1465) have a cross-sectional design. At follow-up, renal status was verified by a review of all available medical files, including laboratory data (Study II, N=2304), and data on CAD events were retrieved from the Finnish Hospital Discharge Register and the Causes of Death Register (Study IV, N=3520). Ophthalmologic data from fundus photographs and ophthalmic records were graded with the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale (Study III).

Results
Triglycerides and apolipoprotein (Apo) B were independently associated with estimated glomerular filtration rate (eGFR) in the multivariate models. The recommended lipid concentrations of current treatment guidelines were poorly met, especially regarding the target for LDL cholesterol. Triglycerides and ApoB were independent predictors of progression to micro- and macroalbuminuria, and total cholesterol was an independent predictor of progression to end-stage renal disease. HDL and HDL_2 cholesterol were independently associated with proliferative diabetic retinopathy (PDR), and triglycerides and triglyceride/HDL cholesterol ratio with mild non-proliferative diabetic retinopathy (NPDR). In patients with moderate to severe NPDR or PDR, the correlations between albumin excretion rate (AER) and lipid variables were strong. However, in patients without retinopathy no significant correlations were observed. In multivariate models, ApoB, triglycerides, non-HDL cholesterol, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio were the strongest lipid predictors of an incident CAD event.

Conclusions

Lipid abnormalities were associated with an increased risk of all three diabetic complications studied, i.e. diabetic nephropathy, retinopathy, and incident CAD events. Triglycerides and ApoB were independently associated with AER and eGFR and predicted the progression to micro- and macroalbuminuria as well as incident CAD events. Far lower concentrations of triglycerides than the currently recommended cut-off level (<1.7 mmol/l) increased the risk of progression of renal disease and predicted incident CAD events. Total and LDL cholesterol were poor predictors of an incident CAD event in patients with normal AER, in patients with HbA_{1c} below the median of the cohort, and in women, in whom the ratios of atherogenic and anti-atherogenic lipoproteins and lipids performed better. Current treatment recommendations may need to be revised to reflect residual CAD risk in patients with type 1 diabetes.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Tausta

Sydän- ja verisuonitaudit ovat yleisin kuolinsyy tyypin 1 diabeetikoilla (1), ja ennenaikainen kuolleisuus on erityisen suuri potilailla, joilla on diabeettinen munuaistauti eli nefropatia (2, 3). Diabetekseen liittyvä silmäsairaus, retinopatia, on sokeutumisen yleisin syy länsimaiden työikäisessä väestössä (4). Riskitekijöiden varhainen tunnistaminen ja tehokas hoito ovat ratkaisevassa asemassa jotta voisimme vähentää diabeettisten liitännäissairauksien syntyä.

Tavoitteet

Tavoitteina oli tutkia veren rasva-arvojen ja diabeettisen nefropatian, retinopatian, ja sepelvaltimotautitapahtumien (ensimmäinen sydäninfarkti, sepelvaltimoiden pallolaajennus tai ohitusleikkaus) yhteyttä suuressa valtakunnallisessa tyypin 1 diabetes-populaatiossa.

Aineisto ja menetelmät

Tutkimukset ovat osa FinnDiane-tutkimusta (Finnish Diabetic Nephropathy Study), jonka tavoitteena on selvittää tyypin 1 diabeteksen liitännäissairauksien geneettisiä ja kliinisiä riskitekijöitä. Osatyöt I (N = 2927) ja III (N = 1465) ovat poikkileikkaustutkimuksia. Seurantatutkimuksissa tieto munuaistaudin vaikeusasteesta varmennettiin kaikista käytettävissä olevista sairaskertomuksista (osatyö II, N = 2304), ja tiedot sepelvaltimotapahtumista saatiin hoitoilmoitusjärjestelmästä (HILMO) sekä kuolinsyyrekisteristä (osatyö IV, N = 3520).

Tulokset

Hoitosuositusten mukaiset kolesterolipitoisuudet ylittyivät monella potilaalla ja erityisen huonosti toteutuivat LDL-kolesterolin suositukset. Kohonneet triglyseridi- ja apolipoproteiini (Apo) B-pitoisuudet ennustivat varhaisen nefropatian (mikroalbuminuria) sekä nefropatian (makroalbuminuria) kehittymistä. Korkea kokonaiskolesteroli oli itsenäinen riskitekijä loppuvaiheen munuaistaudin kehittymiselle. Matalat HDL- ja HDL_2-kolesterolipitoisuudet olivat yhteydessä proliferatiiviseen diabeettiseen retinopatiaan (PDR), ja koholla oleva triglyseridipitoisuus sekä triglyseridi/HDL-kolesterolisuhde olivat yhteydessä lievään taustaretinopatiaan (NPDR). Potilailla, joilla oli kohtalainen tai vaikea NPDR tai PDR, korrelaatiot veren rasvojen ja virtsan albumiinierityksen (AER) välillä olivat vahvoja, mutta vastaavia korrelaatiota ei havaittu lainkaan potilailla, joilla ei ollut merkkejä retinopatiasta. ApoB, triglyseridit, ei-HDL-kolesteroli, ApoB/ApoA-I-suhde sekä triglyseridi/HDL-kolesterolisuhde olivat vahvimpia sepelvaltimotautitapahtuman ennustajia tyypin 1 diabeetikoilla.

Päätelmät

Epäsuotuisat veren rasva-arvot olivat yhteydessä kaikkiin kolmeen tutkittuun diabeteskomplikaatioon, eli diabeettiseen nefropatiaan, retinopatiaan, ja sepelvaltimotautiin. Koholla olevat triglyseridi- ja ApoB-pitoisuudet ennustivat munuaistaudin etenemistä sekä sepelvaltimotautitapahtumaa. Jo nykyisiä suosituksia (<1.7 mmol/l) huomattavasti matalampi triglyseridipitoisuus ennusti munuaistaudin etenemistä ja sepelvaltimotautitapahtumia. Kun potilaat jaettiin ryhmiin sukupuolen, munuaistaudin tai sokeritasapainon mukaan, kokonais- ja LDL-kolesteroliarvot eivät olleet yhteydessä sepelvaltimotautitapahtumaan naisilla, potilailla joilla oli normaali AER, eikä potilailla joilla HbA_{1c} oli alle 8.3 %. Näillä potilailla aterogeenisten ja anti-aterogeenisten lipoproteiinien suhteet olivat parempia sepelvaltimotautitapahtuman ennustajia. Nykyiset hoitosuositukset tulisi tarkistaa, jotta mahdollinen suurentunut sepelvaltimotautiriski tyypin 1 diabeetikoilla havaittaisiin paremmin.

ABSTRAKT (ABSTRACT IN SWEDISH)

Bakgrund
Hjärt-och kärlsjukdomar är den vanligaste dödsorsaken bland typ 1 diabetiker (1), och den förtidiga dödligheten är särskilt hög hos patienter med diabetisk njursjukdom, nefropati (2, 3). Diabetisk ögonsjukdom, retinopati, är den vanligaste orsaken till blindhet bland den arbetsföra befolkningen i västvärlden (4). En tidig identifiering och effektiv behandling av riskfaktorer har en avgörande betydelse för att minska förekomsten av följdsjukdomar vid typ 1 diabetes.

Studiens målsättningar
Att undersöka sambandet mellan lipidprofiler och diabetisk nefropati, retinopati och kranskärlssjukdom (första hjärtinfarkt, ballongutvidgning eller bypassoperation av hjärtats kranskärl) i en stor landsomfattande kohort av patienter med typ 1 diabetes.

Patienter och metoder
Dessa studier är en del av den pågående FinnDiane-studien (Finnish Diabetic Nephropathy Study), en landsomfattande, multicenterstudie vars målsättning är att identifiera både genetiska och kliniska riskfaktorer för utvecklingen av följdsjukdomar vid typ 1 diabetes. Studie I (N = 2927) och III (N = 1465) var tvärsnittsstudier. Vid uppföljningen verifierades progression av njursjukdom genom en granskning av alla tillgängliga sjukjournaler (studie II, N = 2304) och uppgifter om kranskärlssjukdom söktes ur Finlands patient- och dödsorsaksregister (studie IV, N = 3520).

Resultat
De rekommenderade lipidvärdena i gängse behandlingsöversikter uppfylldes dåligt, speciellt gällande målsättningen för LDL-kolesterolnivån. Förhöjda triglycerid- och apolipoprotein (Apo) B-nivåer var oberoende riskfaktorer för utveckling av begynnande njursjukdom (mikroalbuminuri) samt progression till nefropati (makroalbuminuri). Förhöjda totalkolesterolkoncentrationer var en oberoende riskfaktor för progression till terminal njursvikt. Låga HDL- och HDL_2-kolesterolnivåer var associerade med proliferativ diabetisk retinopati (PDR), och triglycerider samt triglycerid/HDL-kolesterolförhållandet med mild icke-proliferativ diabetisk retinopati (NPDR). Hos patienter med måttlig till svår NPDR eller PDR, var korrelationerna mellan lipider och albuminutsöndring i urinen (AER) starka, men bland patienter utan retinopati kunde inga signifikanta korrelationer observeras. De starkaste lipidprediktorerna för insjuknandet i kranskärlssjukdom hos typ 1 diabetiker var ApoB, triglycerider, icke-HDL-kolesterol, ApoB/ApoA-I-förhållandet samt triglycerid/HDL-kolesterolförhållandet.

Slutsatser

En ofördelaktig lipidprofil var associerad med samtliga av de tre undersökta diabeteskomplikationerna, dvs. diabetisk nefropati, retinopati, och kranskärlssjukdom. Förhöjda triglycerid- och ApoB-nivåer förutspådde både progression av njursjukdom samt insjuknande i kranskärlssjukdom. Betydligt lägre koncentrationer av triglycerider än den för tillfället rekommenderade nivån (<1.7 mmol/l) ökade risken för progression av njursjukdom och insjuknande i kranskärlssjukdom bland typ 1 diabetiker. När patienterna delades in i grupper på basen av kön, graden av njursjukdom eller sockerbalans, förutspådde inte total- och LDL-kolesterolnivåerna kranskärlssjukdom hos kvinnor, patienter med normalt AER, eller patienter med HbA_{1c} under 8.3 %. Förhållandet mellan de aterogena och anti-aterogena lipiderna var betydligt bättre prediktorer för kranskärlssjukdom hos dessa patienter. Gängse behandlingsrekommendationer för typ 1 diabetiker bör eventuellt revideras för att bättre upptäcka den potentiellt ökade kranskärlssjukdomsrisken.

1 INTRODUCTION

Diabetes is one of the most common chronic diseases worldwide, and in 2013 altogether 382 million people were estimated to have diabetes (5). The global prevalence of type 2 diabetes is increasing in epidemic proportions due to an increase in obesity, a low level of physical activity, and aging of the population. In 2035 a predicted 592 million people will have diabetes (5). In Finland, ~250 000 individuals have diagnosed type 2 diabetes and ~200 000 are estimated to have undiagnosed type 2 diabetes (6). It is noteworthy that Finland has the highest incidence of type 1 diabetes in the world (7), with the current number of patients with type 1 diabetes being ~50 000 (8). The incidence of type 1 diabetes is also increasing worldwide, but the reasons for this remain unclear (9).

Type 1 diabetes was a fatal disease until the discovery of insulin in 1921. Despite modern insulin treatment, glycemic control is poor in many patients with type 1 diabetes, and in the Finnish Diabetic Nephropathy Study (FinnDiane) cohort only ~15% of the patients had reached the recommended glycosylated hemoglobin A_{1c} (HbA_{1c}) level of <7.0% (10). With increasing HbA_{1c}, the frequency of diabetic complications also increases substantially. Diabetic complications lead to reduced quality of life and premature death. The management and treatment of these complications also cause an immense economic burden (11).

Diabetic kidney disease (nephropathy) is the leading cause of dialysis or kidney transplantation (12). Nephropathy develops in about one-third of patients with type 1 diabetes, and the highest incidence peak is seen after 15-20 years of diabetes (13, 14). Diabetic eye disease (retinopathy) is the most common cause of blindness among the working-aged population in the Western world (4). Proliferative diabetic retinopathy (PDR), the advanced form of diabetic retinopathy, occurs in around 40% of patients with type 1 diabetes after 25 years of diabetes duration (15). Diabetic nephropathy and retinopathy share several risk factors and are strongly associated with each other (16). The studies regarding the association between lipid variables and retinopathy have yielded conflicting results (17-20), and the effect of renal disease on this relationship is unclear. Also unknown is how retinopathy status affects the association between albumin excretion rate (AER) and lipid variables.

The most common cause of death in patients with type 1 diabetes is cardiovascular disease (CVD) (21). The increased incidence of CVD in patients with type 1 diabetes is mostly related to renal disease, and the mortality rates of patients with type 1 diabetes without any signs of renal disease are comparable with those of the general population, whereas in patients with end-stage renal disease (ESRD) an 18- to 30-fold increase in mortality has been observed (2, 3). The role of the lipid variables in the development of coronary artery disease (CAD) has been studied thoroughly in the general population and in patients with type 2 diabetes, but studies in patients with type 1 diabetes are scarce.

The main aim of these series of studies was to evaluate the relationship between lipid profiles and different diabetic complications, i.e. diabetic nephropathy, retinopathy, and incident CAD events, in a large nationwide cohort of patients with type 1 diabetes.

2 REVIEW OF THE LITERATURE

2.1 Types of diabetes mellitus

2.1.1 Definition of diabetes

Diabetes mellitus is a chronic systemic disease characterized by an increased blood glucose concentration. The word diabetes is derived from the Greek word "diabainein" and means "to pass through", referring to the large volume of urine, while mellitus comes from the Latin term "mel", which means honey and refers to the sweetness of the urine from patients with untreated diabetes. Diabetes is caused by either decreased production of insulin from the pancreatic β-cells or decreased effect of insulin on target tissues or by a combination of these two. Diabetes not only causes disturbances in carbohydrate metabolism, but also affects lipid and protein metabolisms. Diabetes is defined as an increased fasting plasma glucose ≥ 7.0 mmol/l or a 2-h plasma glucose ≥ 11.1 mmol/l during an oral glucose tolerance test or HbA_{1c} ≥ 6.5% or a random plasma glucose of ≥ 11.1 mmol/l in a patient with classic symptoms of hyperglycemia (thirst, weight loss, and polyuria) (22-24).

2.1.2 Classification of diabetes

The two major categories of diabetes are type 1 and type 2 diabetes, previously also called "insulin-dependent" (IDDM) and "non-insulin-dependent" (NIDDM), or "juvenile" and "adult-onset" diabetes, respectively. Type 1 diabetes is characterized by an autoimmune reaction that leads to a total loss of function of the insulin-secreting β-cells of the islets of Langerhans in the pancreas, resulting in absolute insulin deficiency. Type 2 diabetes is the consequence of decreased insulin sensitivity (primarily in skeletal muscles, adipose tissue, and liver) and/or decreased insulin secretion from β-cells. It is the most common form of diabetes and is increasing in epidemic proportions worldwide. Considerable overlap exists between the two conditions, and type 1 and type 2 diabetes have been proposed to be different forms of the same disease, the main difference being the absence of an immune response in patients with type 2 diabetes, leading to a slower rate of β-cell loss (25). On the other hand, the clear lack of evidence for similar genetic factors predisposing to type 1 and type 2 diabetes supports the notion of two separate diseases.

Latent Autoimmune Diabetes of the Adult (LADA) is classified as a form of type 1 diabetes and is characterized by the presence of islet autoantibodies, most typically glutamic acid decarboxylase antibodies (GADA), leading to the destruction of pancreatic β-cells (26, 27). Patients with LADA are usually older than the patients with type 1 diabetes and do not require insulin at the time of diagnosis, but after 5 years of diabetes

duration ~80% require insulin treatment. The clinical phenotype resembles that of type 2 diabetes, and LADA patients may initially be diagnosed as having type 2 diabetes. Due to the features described above, LADA is sometimes also called "type 1.5 diabetes" or "slow-onset type 1 diabetes".

Maturity onset diabetes of the young (MODY) is a heterogeneous group of disorders caused by mutations in different autosomal dominant genes with high penetration, affecting insulin production or insulin release from pancreatic β-cells (28). It can also be referred to as monogenic diabetes, in contrast to the more complex type 1 and type 2 diabetes, which involve multiple genes with low penetration as well as environmental factors. MODY patients do not display the β-cell autoimmunity or ketoacidosis experienced by patients with type 1 diabetes, and the age at onset is usually younger than in patients with type 2 diabetes. Typical characteristics of type 1 and type 2 diabetes, LADA, and MODY can be seen in Table 1.

Table 1. Typical characteristics of different forms of diabetes.

	Type 1 diabetes	**Type 2 diabetes**	**LADA**	**MODY**
Age at onset	Usually age below 35 years (highest peak at puberty)	Usually in adults over 40 years (also in obese children/ adolescents)	Usually age above 30 years	Usually age below 30 years
Characteristics of diagnosis	Acute, often ketosis	Often insidious	Symptoms develop more slowly than in type 1 diabetes	Variable, many are asymptomatic
Insulin level at diagnosis	Undetectable or very low	High	Low	Variable
Presence of insulin resistance	Usually no (but may be present in obese patients)	Yes	Yes (in some studies less common than in type 2 diabetes)	No (insulin resistance is extremely rare)
Insulin therapy	Essential and permanent	May occur	Usually not at diagnosis, most need insulin within 5 years	May occur (insulin doses are low)
Auto-antibodies	IAA GADA, ICA, IA2-A, ZnT8A…	None	Mostly GADA and ICA (IA-2A, IAA, ZnT8A may be detected)	None/Rare
Genetics	Polygenic	Polygenic	Polygenic	Monogenic
Autoimmune etiology	Yes	No	Yes	No

IAA=insulin autoantibodies, GADA=glutamic acid decarboxylase antibodies, ICA=islet cell antibodies, IA2-A=insulinoma-associated autoantigen 2 antibodies, ZnT8A = Zink transporter 8 antibodies.

Gestational diabetes is diagnosed when hyperglycemia is first recognized during pregnancy and is associated with insulin resistance and increased risk of type 2 diabetes (29).

Secondary forms of diabetes can be caused by, for example, pancreatitis, surgery, pancreatic trauma, and pancreatic cancer. Further, long-term use of such medications as steroids, antipsychotics, and a range of immunosuppressive agents may induce the development of diabetes (30). Medications widely used for prevention of CVD, e.g. thiazide diuretics, beta-blockers, and statins have also been found to be weakly diabetogenic (30, 31).

2.1.3 Epidemiology of type 1 diabetes

Type 1 diabetes is one of the most common chronic diseases of childhood, and in contrast to other autoimmune diseases, it has a male predominance (32). The highest incidence of type 1 diabetes is found in Finland (64 per 100 000 per year below the age of 15) (7), followed by the Italian island of Sardinia and Sweden (33). The lowest incidence of type 1 diabetes is found in Venezuela and China, with only around 0.1 per 100 000 per year (33). The incidence of type 2 diabetes is increasing rapidly, mainly because of sedentary lifestyle and an increase in obesity, but the reasons behind the worldwide increase in the incidence of type 1 diabetes remain unclear. In Finland, a non-linear increase has been observed, and the incidence rate of type 1 diabetes in Finnish children has doubled from 1980 to 2005 (7). Recent studies have reported a plateau in the incidence rates in Finland and Sweden (34, 35). Another recent finding is that the onset of diabetes has shifted towards a younger age (36, 37), and according to the "spring harvest theory" the increasing incidence in children might be compensated by a decrease in the incidence in the older age groups (38). In concordance with this theory, the overall cumulative incidence of type 1 diabetes before the age of 39 years in Sweden and Belgium has remained constant (37, 39). However, in Finland the increasing incidence of type 1 diabetes is also seen in the age group of 15-39 years (40); thus, in Finland the "spring harvest theory" does not seem to apply. Genetic variation is thought to be one explanation for the marked geographical differences in the incidence rates, but genetic changes and more children being borne to parents with type 1 diabetes are still insufficient to explain the increased incidence. Thus, environmental factors must play a role. In support of this, increased incidence of type 1 diabetes has also been reported in children of migrants who have moved from a region of low to high incidence of type 1 diabetes (41, 42).

2.1.4 Pathogenesis of type 1 diabetes

Type 1 diabetes involves selective β-cell loss and is the result of an autoimmune reaction. T-cells (CD8+ and CD4+), macrophages (CD68+), and B-lymphocytes (CB20+) are frequently found in insulitis lesions (43). Autoantibodies against β-cell autoantigens, e.g. insulin autoantibodies (IAA), GADA, islet cell antibodies (ICA), insulinoma-associated autoantigen 2 antibodies (IA-2A), and Zink transporter 8 antibodies (ZnT8A) (44), are found in more than 90% of patients with newly diagnosed type 1 diabetes and they are

already present months to years before symptomatic onset. However, only 25-50% of children with autoantibody positivity will eventually develop clinical type 1 diabetes, hence, many remain in a subclinical state or the β-cell autoimmunity is aborted (45). Type 1 diabetes is a polygenic disease, and thus far, over 40 loci are known to affect susceptibility to the disease (46, 47). Most of these loci are believed to involve immune responses. The highest risk is associated with the human leukocyte antigen (HLA) region on chromosome 6p21 (48), which accounts for ~50% of the genetic susceptibility. However, the proportion of high-risk HLA genotypes in newly diagnosed patients has decreased, and therefore, the influence of the environment is thought to have increased (49). The true triggers of the autoimmune reaction in genetically susceptible individuals remain obscure. High birth weight and weight gain in infancy have been suggested as risk factors for type 1 diabetes, and in the "accelerator hypothesis" increased body mass is thought to overload the β-cells, with the increased insulin demand accelerating the autoimmune attack (25). Different dietary factors, such as cow milk (50), potatoes infested by Streptomyces species (51), gluten (52), and short duration of breast feeding (53), have been suggested as environmental agents initiating the disease process. Other triggers might be vitamin D deficiency (54, 55), enteroviruses (56), Cesarean section (57), and gut microbiota (58). Interestingly, seasonal changes in incidence rates have also been noted. Being born in the spring is associated with a higher risk (59), and more cases are diagnosed in the fall and winter (60) at the peak occurrence of enteroviruses and vitamin D deficiency. The "hygiene hypothesis" suggests that a decrease in infections during childhood leads to inadequate functioning of the immune system (61). In support of this theory, a reciprocal trend has been seen between the incidence of infectious diseases and the incidence of autoimmune and allergic diseases. Furthermore, the incidence of type 1 diabetes is positively associated with the gross national product (62) and is lower in poor countries with a higher population density (63). Also, a protective effect of exposure to infections with early daycare attendance has been observed (64). Interestingly, metabolite and lipid profiles have also been suggested as markers for the development of type 1 diabetes. For example, reduced phosphatidylcholine at birth, and decreased triglycerides and antioxidant ether phospholipids during the follow-up period were observed in children who developed diabetes (65). Further, increased levels of proinflammatory lysophosphatidylcholines were seen months before seroconversion to autoantibody positivity. Increased odd-chain triglycerides as well as polyunsaturated fatty acid-containing phospholipids and lower concentrations of methionine have also been observed in autoantibody-positive children (66).

2.2 Diabetic complications

2.2.1 Diabetic nephropathy

2.2.1.1 Definition

Diabetic nephropathy is defined as a progressive increase in urinary albumin excretion rate (AER), and a decline in glomerular filtration rate. AER is measured from a timed urine collection (either overnight or 24 h). Microalbuminuria is defined as an increase in AER of ≥20 μg/min or ≥30 mg/24 h. Macroalbuminuria (also called proteinuria or overt nephropathy) is defined as an increase in AER of ≥200 μg/min or ≥300 mg/24 h. Several factors can falsely increase AER, e.g. infections, fever, physical exercise, pregnancy, hematuria, menstruation, congestive heart failure, marked hyperglycemia, or hypertension (67). Due to variability in AER, at least two out of three consecutive urine collections are required to define the renal status. Another method for screening is a spot urine sample from which the albumin-to-creatinine ratio (ACR) is measured. The cut-off points for micro- and macroalbuminuria if ACR is used are ≥2.5 or ≥3.5 mg/mmol and >25 or >35 mg/mmol in men and women, respectively. The final stage of diabetic nephropathy is end-stage renal disease (ESRD), defined as requiring dialysis or a renal transplant.

2.2.1.2 Renal function

Renal function, i.e. glomerular filtration rate (GFR), is classified into five categories by the Kidney Disease: Improving Global Outcomes (KDIGO): stage 1 (normal) = GFR ≥90, stage 2 (mildly reduced) = GFR 60-89, stage 3 (moderately reduced) = GFR 30-59, stage 4 (severely reduced) = GFR 15-29, and stage 5 (renal failure) = GFR <15 ml/min/1.73 m^2 (68). GFR can be directly measured by the plasma clearance of inulin or the chromium EDTA method (Cr^{51}-EDTA) (69, 70). Unfortunately, the direct measurement of GFR is laborious and costly, and therefore, not feasible in the routine clinical setting or in studies with large cohorts. Thus, different mathematical formulas have been developed to calculate the estimated GFR (eGFR). The most often used creatinine-based formulas are the Cockcroft-Gault (71), the Modification of Diet in Renal Disease (MDRD) (72), and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (73) (see Discussion in Section 7.5). Creatinine is a waste product of skeletal muscle, and muscle mass therefore influences creatinine production. It is filtered and mainly secreted by the glomerulus, however, there is also tubular secretion of creatinine. Cystatin C is produced at a constant rate and filtered by the kidneys without active tubular secretion, and it has therefore been suggested as an earlier marker of renal dysfunction than creatinine (74). However, no consensus exists as to whether or not cystatin C should replace creatinine measurements. Equations using both creatinine and cystatin C have also been proposed (75, 76).

2.2.1.3 Epidemiology

Diabetic nephropathy is the key determinant of morbidity and mortality in patients with diabetes (2, 77). It is associated with high CVD risk and is also the most common cause of renal failure in the Western world (12). As diabetes is constantly increasing worldwide, renal failure is also becoming a growing healthcare problem. The peak incidence of nephropathy occurs after a 15- to 20-year duration of diabetes (13). It has previously been shown that around one-third of patients with type 1 diabetes will eventually develop diabetic nephropathy (78), but more recent studies have demonstrated that the incidence of diabetic nephropathy has decreased, probably because treatment of the major risk factors has improved (79).

Microalbuminuria is still the best non-invasive predictor of diabetic nephropathy. In patients with diabetes duration of over 15 years, 28% progressed to macroalbuminuria during a 10-year follow-up (80), whereas in earlier studies up to 80% progressed (81, 82). Regression from microalbuminuria to normal AER is not unusual and has varied from around 30% (13, 80) to as high as 58% in one study (83). Initially, it was thought that in patients with microalbuminuria decline in GFR does not occur (apart from the yearly decline in GFR that results from aging, which is ~1 ml/min/year in individuals over 40 years of age). However, in a cohort from the Joslin Diabetes Center a progressive decline in GFR estimated by cystatin C was observed in 31% of patients with microalbuminuria (84).

After the onset of macroalbuminuria, the average GFR decline is around 10-12 ml/min/year without treatment of hypertension, and hence, the development of ESRD would take around 8-10 years (85, 86). Fortunately, efficient treatment of hypertension will slow down the development of ESRD and even regression from macroalbuminuria to normal AER is possible. In a Finnish study, the cumulative incidence of ESRD was lower than previously reported, 7.8% after 30 years of diabetes duration, and the prognosis of type 1 diabetes was more favorable in patients diagnosed in the more recent years (87).

The increase in AER and the decrease in GFR do not always go hand in hand. In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort the cumulative incidence of stage 3 chronic kidney disease (CKD, eGFR <60 ml/min/1.73 m^2) was 11.4%, and out of these patients 24% were normoalbuminuric, 16% microalbuminuric, and 61% macroalbuminuric (88). The decline in eGFR was 1.2%/year and 5.7%/year in normoalbuminuric and macroalbuminuric patients, respectively.

2.2.1.4 Pathogenesis

Diabetic nephropathy in patients with type 1 diabetes leads to morphological changes in renal arterioles, tubules, interstitium, and, most importantly, the glomerulus. Glomerulopathy, with thickening of the glomerular basement membrane and mesangial expansion, is a hallmark of diabetic nephropathy (89). Glomerular basement membrane thickening occurs first and can be observed as early as 2 years after the onset of diabetes (90). Mesangial expansion, mostly due to an increase in the mesangial matrix, can be observed already after 5-7 years of diabetes onset (91). Diffuse mesangial expansion is associated with the pathognomonic nodular lesions called Kimmelstiel-Wilson nodules (92). Glomerular and tubular basement membrane thickening as well as mesangial expansions are consequences of increased accumulation of extracellular matrix components, e.g. type IV collagen, fibronectin, and laminin (93, 94). Different stages of mesangial expansion and the development of Kimmelstiel-Wilson nodules result in glomerulosclerosis (95). Arteriolar hyalinosis, i.e. exudative lesions in which plasma proteins (e.g. immunoglobulins, fibrinogen, and albumin) may ultimately replace smooth muscle cells, may be present a few years after onset (91, 96). Abnormalities of the glomerular-tubular junction are a late manifestation of the disease (97). In advanced diabetic nephropathy, also tubulointestinal injury, such as inflammation, atrophy, and fibrosis, is observed (98).

It is likely that interactions between several risk factors contribute to the development and progression of diabetic nephropathy. Figure 1 illustrates how hyperglycemia, hypertension, inflammation, and dyslipidemia could lead to the activation of various pathways implicated in the pathogenesis of diabetic nephropathy. Potential pathogenic mechanisms behind lipid-induced renal injury are also discussed in Section 2.3.5.

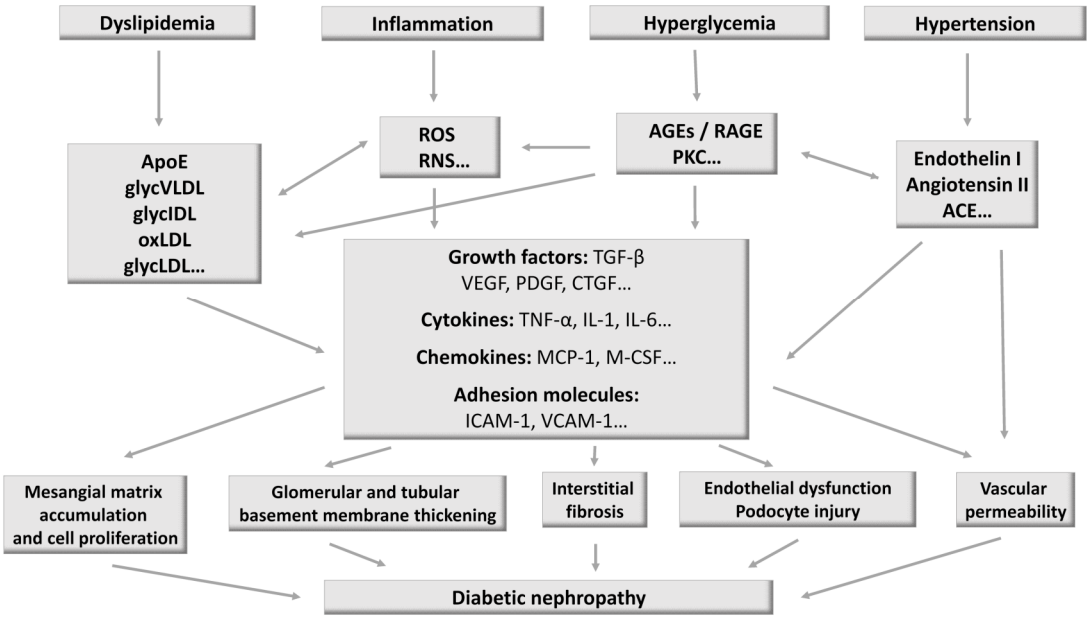


Figure 1. A simplified overview of the various possible pathways implicated in the pathogenesis of diabetic nephropathy. Glyc = glycated, ox = oxidized, ROS = reactive oxygen species, RNS = reactive nitrogen species, AGE = advanced glycation end-products, RAGE = receptor of advanced glycation end-products, PKC = protein kinase C, ACE = angiotensin-converting enzyme, TGF-β = transforming growth factor beta, VEGF = vascular endothelial growth factor, PDGF = platelet-derived growth factor, CTGF = connective tissue growth factor, TNF-α = tumor necrosis factor alpha, IL = interleukin, MCP = monocyte chemotactic protein, M-CSF = macrophage colony-stimulating factor, ICAM = intercellular adhesion molecule, VCAM = vascular cell adhesion molecule.

2.2.1.5 Risk factors

Glycemic control

Long-term glycemic exposure is a prerequisite for the development of diabetic nephropathy, and several studies have demonstrated that poor glycemic control increases the risk of progression of renal disease (99-101). In the Diabetes Control and Complications Trial (DCCT), the development of microalbuminuria was reduced by 39% and progression to macroalbuminuria by 54% in patients with type 1 diabetes with an HbA_{1c} ~7% relative to those with HbA_{1c} ~9% (102). In the post-trial follow-up, the risk of macroalbuminuria was still significantly reduced in the intensively treated group despite similar HbA_{1c} levels after the trial period, suggesting that glycemic memory exists (103). In patients with type 2 diabetes, the United Kingdom Prospective Diabetes Study (UKPDS, HbA_{1c} 7.0% vs. 7.9%), the Action in Diabetes and Vascular Disease (ADVANCE, HbA_{1c} 6.5% vs. 7.3%), and the Veterans Affairs Diabetes Trial (VADT, HbA_{1c} 7.3% vs. 9.3%) have all shown a reduction in microvascular end-points and other beneficial effects of intensified glycemic control (104-106). However, the Action to Control Cardiovascular Risk in Diabetes (ACCORD, HbA_{1c} 6.3% vs. 7.6%) Trial, which

aimed to reduce HbA_{1c} below 6.0%, had to be discontinued after 3.7 years into the trial due to excess mortality in the intensive treatment group (107). Intensive and rapid decrease of the HbA_{1c} can lead to increased risk of hypoglycemia and weight gain, and individualization of treatment goals is thus now emphasized. Large glycemic variability has also been shown to predict progression of both micro- and macroalbuminuria (108, 109).

Blood pressure

Blood pressure increases in parallel with the increase in AER and is positively correlated with the decline in GFR (110-112). Notably, antihypertensive agents, such as Angiotensin-Converting Enzyme (ACE) inhibitors and Angiotensin II Receptor Blockers (ARBs), have shown renoprotective effects independently of their blood pressure-lowering effects (113, 114). For patients with type 1 diabetes, more evidence of the beneficial effect of ACE inhibitors on diabetic nephropathy prevention is available, and recently a meta-analysis also showed that ACE inhibitors reduce all-cause mortality and major CVD events in patients with diabetes (115), whereas no beneficial effects on these parameters were seen with ARB treatment. Therefore, ACE inhibitors are the first line of treatment for blood pressure reduction in patients with type 1 diabetes. However, most patients need multiple antihypertensive agents to reach the blood pressure treatment targets. Dual blockade with both ACE inhibitors and ARBs has been proposed, but it has raised safety concerns and is not widely recommended. Epidemiological analyses have shown that systolic blood pressure (SBP) >120 mmHg predicts the development of ESRD in the long term, and therefore, a treatment goal of <130/80 mmHg was recommended for patients with diabetes (116). However, recent guidelines have changed the treatment goals back to the less stringent goal of <140/80 mmHg due to lack of evidence of beneficial effects with lower SBP targets (117). In the ACCORD Trial, intensified blood pressure treatment (119 vs. 134 mmHg) reduced albuminuria rates and stroke events, but no beneficial effects were seen on other CVD events or renal function (118). In fact, there was an increase in serious adverse events (e.g. syncope and hyperkalemia). A meta-analysis of 14 randomized clinical trials yielded similar results and concluded that an SBP goal between 130 and 135 mmHg is acceptable (119). These trials included patients with type 2 diabetes with a mean age between 55 and 67 years. An SBP target of <130 mmHg may still be appropriate in younger patients, especially if the target can be achieved with fewer drugs and without side-effects.

Insulin resistance

Insulin resistance is not only observed in patients with type 2, but is also a common feature in patients with type 1 diabetes and is observed mainly in the peripheral and hepatic tissues (120). The golden standard for measuring insulin sensitivity is the euglycemic hyperinsulinemic clamp technique (121). Using this technique, insulin resistance was shown to predict the development of microalbuminuria in one study (122), while another found no association between AER and insulin sensitivity (123). Use of the euglycemic hyperinsulinemic clamp is, however, laborious and invasive. Therefore, larger studies have used a surrogate estimate for insulin sensitivity, the estimated glucose

disposal rate (eGDR), which was developed by Williams et al. (124). Notably, in the Pittsburgh Epidemiology of Diabetes Complications (EDC) cohort the eGDR predicted progression to macroalbuminuria (125). Further, in the FinnDiane cohort metabolic syndrome, which is strongly associated with insulin resistance, predicted renal disease progression (126), and having a first-degree relative with type 2 diabetes increased the risk of diabetic nephropathy (127).

Genetics

Diabetic nephropathy clusters in families (128), and in genetically similar patient groups (e.g. Pima Indians, Mexican Americans, Asians, New Zealand Maoris, and Australian Aborigines), the development of diabetic nephropathy is much more common than in individuals of white European origin (129, 130). Therefore, it is likely that genetic factors cause susceptibility to the development of diabetic nephropathy. However, thus far, the results have been a bit disappointing, and very few specific associations between gene variants and diabetic nephropathy have been found. Genes suggested but not shown to be conclusively associated with diabetic nephropathy include e.g. *angiotensin-converting enzyme (ACE), engulfment and cell motility 1 (ELMO1), vascular endothelial growth factor (VEGF), apolipoprotein E (APOE), apolipoprotein C-I (APOC-I), and erythropoietin (EPO).* To date the largest genome-wide association study, including the FinnDiane cohort, is the Genetics of Nephropathy - an International Effort (GENIE) Consortium, which identified an intronic single-nucleotide polymorphism (SNP) in the *v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (ERBB4)* gene to be nominally associated with diabetic nephropathy (131). While this association was not of genome-wide significance, the effect was consistent in all cohorts. It is of note that *ERBB4* encodes a tyrosine kinase receptor and is involved in tubular development (132).

Smoking

Smoking has been shown to increase the risk of development and progression of diabetic nephropathy in patients with type 1 or type 2 diabetes (133). The pathogenic mechanism is thought to be a deleterious effect of smoking on vascular endothelial cells.

Other risk factors

Other suggested risk factors for diabetic nephropathy are e.g. long diabetes duration (134), male sex (13), anemia (135, 136), low birth weight (137), short adult stature (138), high protein diet (139), adiponectin (140), advanced glycation end-products (AGEs) (141), inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP) (142), and lipid variables. The role of the lipid profile in the development of diabetic nephropathy is discussed at length in Sections 7.1 and 7.2. Several studies have explored the associations between lipid variables and renal disease (143-146); however, whether the lipid abnormalities precede or occur concomitantly with the increase in AER is still under debate. Furthermore, how retinopathy status affects the association between AER and lipid variables is also unknown.

2.2.2 Diabetic retinopathy

Diabetic retinopathy is a feared complication and the leading cause of adult onset blindness in the working-aged population in the Western world (4). The advanced form of diabetic retinopathy, proliferative diabetic retinopathy (PDR), is sight-threatening and characterized by the proliferation of abnormal new, fragile blood vessels in response to ischemia and vitreous hemorrhage (Figure 2). PDR is preceded by non-proliferative diabetic retinopathy (NPDR), which is classified into different stages depending on the presence and severity of microaneurysms, hemorrhages, retinal edema, lipid exudates, intraretinal microvascular abnormalities (IRMAs), and microinfarcts. A detailed classification of diabetic retinopathy was developed by the Early Treatment of Diabetic Retinopathy Study (ETDRS) (147) in the 1980s and it has become the golden standard to define the severity of diabetic retinopathy.

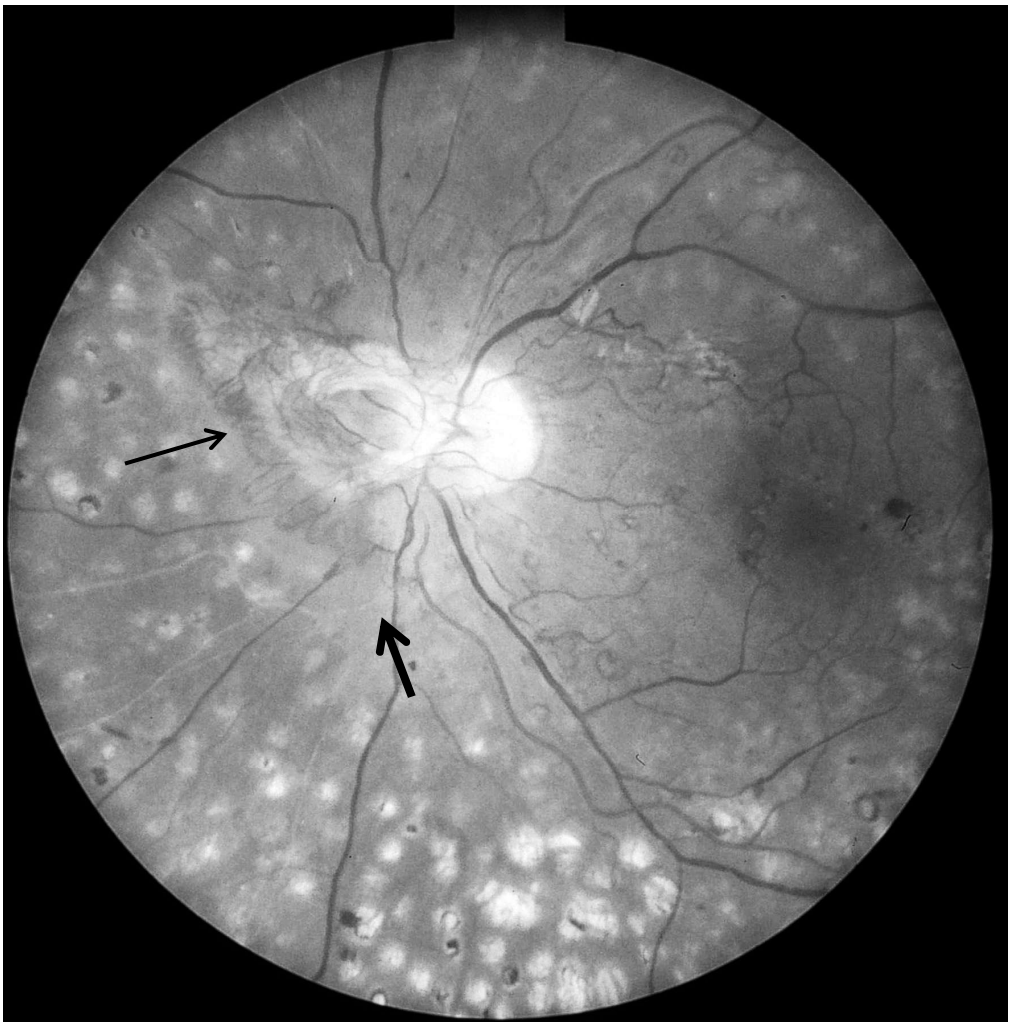


Figure 2. Fundus photograph of proliferative diabetic retinopathy with neovascularization (thin arrow) and an occluded artery (thick arrow) as well as scatter laser scars.

Diabetic macular edema involves leakage and exudation at the center of the eye (macula) and may lead to impairment of central vision (Figure 3). Macular edema is more common in elderly patients and therefore also more common in patients with type 2 diabetes. It can be divided into mild (some retinal thickening or hard exudates in the posterior pole, but distant from the center of the macula), moderate (retinal thickening or hard exudates approaching the center of the macula, but not involving the center), and severe (retinal thickening or hard exudates involving the center) (148). Macular edema is generally asymptomatic at early stages, but if it progresses it can cause severe visual disability, especially in older patients (149). It is the most common cause of visual loss in patients with type 2 diabetes (150).

Figure 3. Fundus photograph of macular edema with hard exudates (=lipid breakdown products due to vascular leakage=thin arrow) and intraretinal hemorrhages (thick arrow).

2.2.2.1 Epidemiology

With a sufficiently long duration of diabetes, nearly all patients with type 1 diabetes will eventually have some degree of diabetic retinopathy (15, 151), however, the rate of the progression and the severity of retinopathy varies substantially. The most profound increase in the incidence of PDR is usually seen after a 10 year duration of diabetes (152).

A number of studies have looked at the prevalence and incidence of diabetic retinopathy, but the results have been very conflicting due to differences in methodology (153). In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), a population-based study including 995 patients with type 1 diabetes, the 25-year cumulative incidence of any retinopathy was 97%, and the incidence of PDR was 42% (15). Due to improved management of diabetes and related risk factors, a reduction in the incidence of retinopathy has been noted in recent studies. In a meta-analysis including patients with diabetes without ocular treatment for retinopathy at baseline, the 4-year incidence of PDR in studies performed before and after 1985 was 19.5% and 2.6%, respectively (154). Also in the FinnDiane cohort, a reduction in the cumulative incidence of severe diabetic retinopathy over the last decades has been observed (155).

Prompt laser treatment is crucial for the prevention of severe vision loss in patients with PDR (156). However, laser treatment destroys retinal tissue, and the desired result is not always achieved, and sometimes treatment is initiated too late. Therefore, comprehensive screening and treatment and recognition of risk factors are crucial for the prevention of diabetic retinopathy.

2.2.2.2 Risk factors

Hyperglycemia is the most important modifiable risk factor for prevention of diabetic retinopathy. Strong evidence indicates an association between poor glycemic control and worsening of diabetic retinopathy and between improved glycemic control and favorable outcome. The first long-term study conducted for the period 1947-1973 followed 4398 patients with diabetes and showed that poor glycemic control assessed cumulatively over the years is related to a higher incidence and progression of retinopathy (157). In the DCCT Study, intensified glycemic control in patients with type 1 diabetes for a mean of 6.5 years resulted in a 47% reduction of the risk of severe NPDR or PDR (158). Further, intensified treatment reduced the incidence of retinopathy by 76% and slowed down the progression of retinopathy by 54% in patients with retinopathy at baseline. With HbA_{1c} above 6.5%, there is a clear increase in the prevalence of retinopathy, which partly also explains why this threshold has been chosen for the diagnosis of diabetes (23). Paradoxically, a transient worsening of diabetic retinopathy is seen if the glycemic control improves too rapidly, a phenomenon called “early worsening” (159, 160), and similarly as for the diabetic nephropathy progression, large variability of the HbA_{1c} was a risk factor for diabetic retinopathy in both the DCCT (108) and the FinnDiane cohort (161).

Elevated blood pressure is associated with the progression of diabetic retinopathy in patients with type 1 diabetes (162), and antihypertensive treatment is associated with slower progression of diabetic retinopathy. In the UKPDS Trial, including hypertensive patients with type 2 diabetes, tighter blood pressure control (defined as <150/85 mmHg) resulted in a 35% reduction in the need for laser treatment and 25% less patients with a ≥2-step progression on the ETDRS severity scale (163). In the EURODIAB Study,

treatment with an ACE inhibitor, lisinopril, in patients with type 1 diabetes reduced diabetic retinopathy progression by 50% after only 2 years of follow-up (164). Further, treatment with an ARB, candesartan, also reduced the incidence of diabetic retinopathy in patients with type 1 diabetes (165) and increased regression of diabetic retinopathy in patients with type 2 diabetes (166). However, no additional benefits from intensively lowered blood pressure targets (SBP <120 mmHg) have been found (167).

Duration of diabetes is the strongest non-modifiable risk factor for diabetic retinopathy. After a 5-year duration of diabetes, the prevalence of diabetic retinopathy is only 17%, whereas in those with ≥15 years it is as high as 97.5% (168).

Pregnancy causes a transient increase in the risk of diabetic retinopathy, but fortunately the long-term risk of diabetic retinopathy seems to be unaffected (169, 170).

Smoking has also been associated with the progression of diabetic retinopathy, however, the results have been somewhat inconsistent and suggested to be mediated through the poorer glycemic control observed in smokers (171, 172).

Other risk factors associated with retinopathy are e.g. anemia (173), waist-hip ratio (WHR) (174), recent cataract surgery (175), puberty (176), and heavy alcohol consumption (177). Dyslipidemia has also been suggested to be a risk factor for diabetic retinopathy, but previous studies have yielded conflicting results (17-20). In fact, most studies to date have reported a lack of an association between lipid variables and diabetic retinopathy (178), leading to the conclusion that traditional lipid variables are most likely not related to diabetic retinopathy.

Diabetic nephropathy and retinopathy are strongly associated with each other and share several risk factors and mechanisms of disease progression (16). Micro- and macroalbuminuria are strongly associated with diabetic retinopathy, especially in patients with younger age at onset (179). However, renal status has been unknown or not taken into account in most retinopathy studies, and thus, comparison between studies is problematic when the prevalence of renal disease has most probably varied between the studied cohorts. The interactions between diabetic retinopathy, nephropathy, and lipid variables have also not been studied previously. How these interactions affect the associations between complications and lipid variables is thus unknown.

2.2.3 Diabetic neuropathy

Diabetic neuropathy is the most common form of neuropathy in industrialized countries, and in clinical practice the simple definition of "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" (180) can be used. The prevalence of diabetic nephropathy increases with longer diabetes duration and has been reported to be around 50% after 15-20 years of diabetes (181),

although large variation is present in the prevalence estimates between studies. Screening for neuropathy is recommended 5 years after the onset of type 1 diabetes, after which an annual examination is advised (117). Distal symmetric polyneuropathy (DPN) is the most common form of diabetic nephropathy (182), and clinical tests for its detection include vibration sensation assessment, monofilament perception, and ankle reflex testing. Around one-third of all patients with diabetic neuropathy suffer from unpleasant sensory symptoms such as pain, numbness, burning sensation, or tingling (183). A wide range of medications, e.g. pregabalin, venlafaxine, duloxetine, gabapentin, amitriptyline, valproate, and opioids is used to treat neuropathic pain (184), which is often, however, resistant to treatment. No specific treatment for the underlying nerve damage is available, except improved glycemic control, which has been shown to prevent the development and progression of the disease (185). The symptoms can also be diminished by avoidance of extreme fluctuations in blood glucose levels (117). Up to half of the patients with DPN may be asymptomatic, however, due to loss of sensation they have an increased risk of foot injury. Foot ulcers are often initiated by diabetic neuropathy and together with peripheral arterial disease, neuropathy is the major underlying cause of diabetic foot ulcers and amputation of lower extremities, which cause suffering and disability and are laborious and costly for the healthcare system (186).

Diabetic autonomic neuropathy can cause e.g. exercise intolerance, orthostatic hypotension, gastroparesis, constipation, diarrhea, erectile dysfunction, loss of bladder control, silent ischemia, impaired sweat gland innervation, and decreased response to hypoglycemia. Cardiovascular autonomic neuropathy (CAN) can be detected by changes in heart rate variability and an abnormal response to deep breathing, standing, and the Valsalva maneuver. An advanced stage of CAN is indicated by resting tachycardia (pulse >100/min) and orthostatic hypotension (i.e. a decrease in SBP [20 mmHg] or diastolic blood pressure [DBP, 10 mmHg] after standing up without an appropriate heart rate response) (117). CAN is an independent risk factor for cardiovascular mortality (187), and a multifactorial intervention targeting hyperglycemia, hypertension, smoking, dyslipidemia, and other lifestyle risk factors has been shown to reduce the development and progression of autonomic neuropathy in patients with type 2 diabetes (188).

2.2.4 Macrovascular complications

Cardiovascular disease (CVD) is the major cause of death in patients with type 1 diabetes (21). The main clinical features of CVD are coronary artery disease (CAD), stroke, and peripheral vascular disease. The risk of cardiovascular morbidity and mortality is at least 2- to 4-fold higher in patients with type 2 diabetes than in the general population (189, 190). The risk has previously been considered to be of similar magnitude in patients with type 1 diabetes, but recent studies have shown that the additional mortality risk is strongly connected to the renal status. In the FinnDiane cohort, patients with type 1 diabetes with a normal AER showed no excess in mortality beyond that of the general population, whereas microalbuminuria was associated with a 3-fold, macroalbuminuria with a 9-fold,

and ESRD with an 18-fold increase in all-cause mortality (2). The results have later been replicated in the Pittsburgh EDC cohort with a 20-year follow-up period (3). The reason for the strong association between CVD and diabetic nephropathy remains unknown. The two complications share several risk factors and are thought to develop in parallel, but it can also be argued that CVD develops as a consequence of the hypertension, inflammation, and dyslipidemia caused by diabetic nephropathy.

The most well-known risk factors for both CVD and diabetic nephropathy are microalbuminuria, decrease in GFR, age, duration of diabetes, hypertension, glycemic control, obesity, insulin resistance, smoking, genetic predisposition, and dyslipidemia (130). The reports on hyperglycemia as a risk factor for CVD have been conflicting, and glycemic control may be more important for microvascular than macrovascular complications. However, glycemic control predicts coronary artery calcification (CAC), and in the DCCT/EDIC Study, intensive glycemic treatment was associated with less atherosclerosis in the period after the original trial (191, 192). Moreover, in a Finnish study, an increment in HbA_{1c} of 1% increased CVD mortality by 52.5% (95% confidence interval [CI] 28.4–81.3) in patients with type 1 diabetes and by 7.5% (4.3–10.8) in patients with type 2 diabetes (193).

2.2.4.1 Coronary artery disease

Type 1 diabetes is associated with earlier onset and faster progression of atherosclerosis (1, 194). Carotid intima-media thickness, which is considered a surrogate marker for early atherosclerosis, is increased in patients with type 1 diabetes and is similar to the levels of healthy control subjects who are 20 years older (195). Further, in an earlier study, 35% of patients with type 1 diabetes died of CAD by age 55, compared with only 8% and 4% of non-diabetic men and women, respectively (196). In a more recent study, the standardized mortality rates from CAD were 9 times higher in men and 42 times higher in women with type 1 diabetes under the age of 40 years (197). The protective effect of female sex is lost in women with type 1 diabetes, which leads to the higher standardized mortality rates observed in women in several studies (193, 196, 197). Type 1 diabetes is also associated with a higher frequency of asymptomatic CAD and as many as 24% of asymptomatic patients with type 1 diabetes between 35 and 60 years of age have ischemia on either exercise electrocardiography, 24-h Holter monitoring, or dynamic perfusion scintigraphy (198). CAD in patients with type 1 diabetes is more diffuse, with a higher likelihood of stenosis of all three major coronary arteries in the distal segments (199). The involvement of distal segments renders the patients frequently unsuitable for bypass grafts. The mortality rate of CAD events in patients with diabetes is also higher (200, 201).

Despite lipid variables and CAD events being extensively studied in the general population and in patients with type 2 diabetes, data on patients with type 1 diabetes are surprisingly scarce. Which lipid variable is the best predictor of an incident CAD event and how concomitant renal status affects this relationship are unknown.

2.3 Lipoprotein metabolism

Lipoprotein particles transport non-water-soluble cholesterol and triglycerides in plasma and consist of a hydrophilic surface monolayer of phospholipids, free cholesterol, and apolipoproteins (Apo) and a central core of hydrophobic cholesterol esters and triglycerides. They can be classified into five major classes according to their hydrated density by ultracentrifugation: chylomicrons (density (d) <0.93 g/ml), very-low-density lipoproteins (VLDL, d=0.93-1.006 g/ml), intermediate-density lipoproteins (IDL, d=1.006–1.019 g/ml), low-density lipoproteins (LDL, d=1.019−1.063 mg/dl), and high-density lipoproteins (HDL, d=1.063–1.21 mg/dl) (202).

The largest lipoprotein particles, chylomicrons, are responsible for the transport of dietary triglycerides and cholesterol. They consist mainly of triglycerides (~85-90%), cholesterol esters, phospholipids, and $ApoB_{48}$. $ApoB_{48}$ is intestinally produced and has 48% of the molecular weight of $ApoB_{100}$ (hereafter referred to as ApoB). In addition to $ApoB_{48}$, chylomicrons also contain ApoA-I and acquire ApoC-I, -II, -III, and ApoE from HDL particles. The close interrelationship between the metabolic pathways for HDL particles and triglyceride-rich lipoproteins is illustrated in Figure 4. In the circulation, the triglycerides of the chylomicrons are hydrolyzed by the enzyme lipoprotein lipase (LPL) into triglyceride-poorer particles, i.e. chylomicron remnants, which are taken up by the liver.

VLDL particles are secreted by the liver and compromise endogenous triglycerides (~55%) cholesterol (~25%), phospholipids (~18%), and ApoB, ApoA-II, ApoC-I, -II, -III and ApoE (203). In the circulation, the triglycerides of the VLDL are also hydrolyzed by LPL. During this process phospholipids, ApoC, and ApoE are transferred to HDL particles and the VLDL particles are in turn transformed into IDL particles.

IDL particles contain ApoB and ApoE and lie between the VLDL and LDL particles in their composition (203). IDL particles can be taken up by the liver or they can be further metabolized to LDL particles by the enzyme hepatic lipase (HL).

LDL particles consist of triglycerides (~6%), cholesterol (~55%), phospholipids (~20%), and ApoB and are the main cholesterol-bearing lipoproteins in the plasma (203). Each VLDL-IDL-LDL particle contains only one ApoB molecule, and therefore measuring ApoB works as a marker of the number of these atherogenic lipoprotein particles in the circulation (204). As the plasma residence time of VLDL is ~2-6 h, IDL ~1 h, and LDL ~1.5-3 days (203, 205), about 90% of the circulating ApoB is found in the LDL particles (206).

Lipoprotein(a) (Lp[a]) is an LDL-like lipoprotein, but in addition to ApoB, the glycoprotein apolipoprotein(a) is also attached to the particle. Most people have very low concentrations of Lp(a), but 2- to 4-fold higher concentrations are found in people of African origin (207, 208). Most methods to measure or calculate LDL cholesterol

concentrations do not distinguish between cholesterol derived from LDL or Lp(a), and therefore, the reported LDL cholesterol is the net sum of cholesterol from both lipoprotein particles.

HDL particles are secreted by the liver as small, lipid-poor lipoproteins, containing mostly ApoA-I. HDL particles are in a constant state of lipidation and delipidation and remodeling. ApoA-I constitutes about 70% of the protein content of HDL particles (209), and each HDL particle contains one to five copies of ApoA-I. In addition, ApoA-II, -IV, -V, ApoC-I, -II, -III, and ApoE may be present. All in all, HDL particles compromise over 100 proteins, which are considered to play an important role for the function of the HDL particles (203). Nascent HDL particles receive cholesterol and phospholipids from peripheral cells via the ATP-binding cassette A1 transporter. Within the HDL particles, free cholesterol is esterified by the enzyme lecithin–cholesterol acyltransferase (LCAT), leading to the formation of HDL_3 particles. Thereafter, the phospholipid transfer protein (PLTP) enzyme promotes the fusion of two denser HDL_3 particles, leading to the formation of one, more buoyant HDL_2 particle (210). HDL_2 particles are degraded by HL and endothelial lipase to HDL remnant particles, which are taken up by the liver by the scavenger receptor (211).

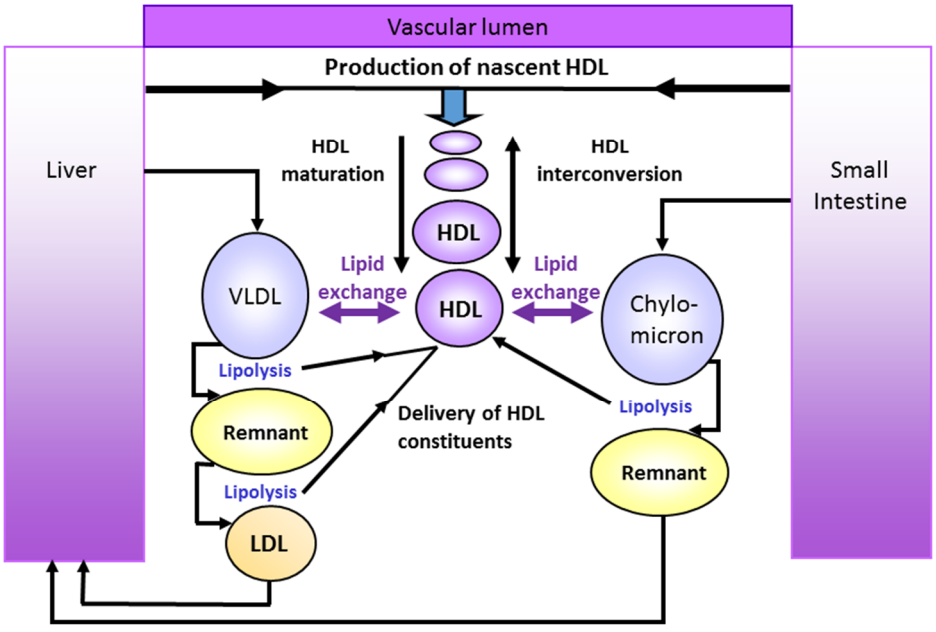


Figure 4. Metabolic pathways for triglyceride-rich lipoprotein remnants and HDL particles (modified from Chapman MJ et al., European Heart Journal 2011) (212).

2.3.1 Actions of insulin on lipoprotein metabolism

By inhibiting the HL, insulin enhances the storage of triglycerides in the adipose tissue as well as reduces the release of free fatty acids from the adipose tissue. Insulin has multiple sites of action on lipid metabolism in the liver (Figure 5). It inhibits VLDL production in the liver (213) and activates LPL in adipocytes (214), which promotes the catabolism of triglyceride-rich lipoproteins (TRLs) (i.e. chylomicrons and VLDL particles). Moreover, insulin decreases ApoB secretion by promoting ApoB degradation in the liver (215) and also enhances the clearance of LDL by increasing the LDL B/E receptor activity (216).

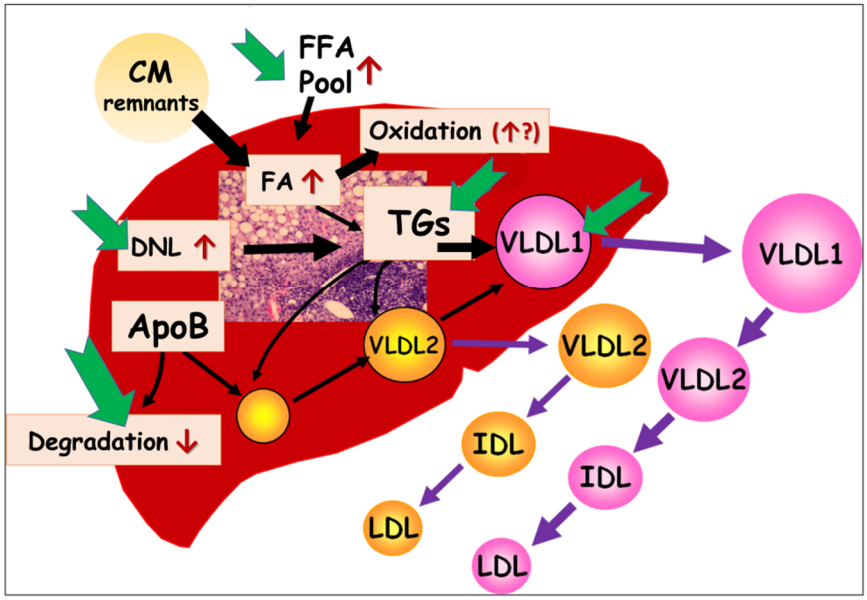


Figure 5. VLDL secretion from the liver is regulated by insulin through several pathways (green arrows) and is increased in insulin-resistant states (red arrows) (modified from Choi SH and Ginsberg HN, Trends in Endocrinology and Metabolism 2011) (217). Insulin suppresses FA oxidation and *de novo* lipogenesis. It also suppresses MTP synthesis, which is a rate-limiting step in hepatic VLDL production. Insulin can also directly affect apolipoprotein (Apo) B secretion by targeting it for degradation, which inhibits VLDL secretion. Expression of ApoC-III, an inhibitor of lipoprotein lipase, is also suppressed by insulin. In insulin-resistant states, there is an increase in the hepatic secretion of VLDL particles due to increased hepatic triglycerides from the enhanced fatty acid flux to the liver, the excess availability of ApoB, and the increased *de novo* lipogenesis. CM = chylomicron, FFA = free fatty acids, FA = fatty acids, DNL = *de novo lipogenesis,* TG = triglycerides.

In patients with newly diagnosed type 1 diabetes with ketoacidosis and insulin deficiency, a reduction of triglyceride-rich lipoprotein catabolism and a profound increase in TRLs, mainly because of decreased LPL activity, can be observed (218, 219). HDL cholesterol concentrations are also significantly decreased as a consequence of hypertriglyceridemia (220). In contrast, in patients with type 1 diabetes and good glycemic control, the triglyceride concentration is usually normal or slightly decreased (218, 219, 221), due to enhanced downregulation of VLDL production by increased plasma insulin concentrations

as a consequence of subcutaneous insulin treatment (218, 222). Further, patients with type 1 diabetes display peripheral hyperinsulinemia, which increases the activity of LPL, thereby lowering triglyceride concentrations (223). Plasma LDL cholesterol is normal or even slightly decreased in patients with intensified insulin treatment as a consequence of decreased VLDL production (223, 224). HDL cholesterol concentrations are normal or slightly increased in patients with good glycemic control (219). The increase in HDL cholesterol may be due to an increase in the LPL/HL ratio (due to increased LPL activity and normal HL activity) (225), again as a consequence of peripheral hyperinsulinemia caused by subcutaneous insulin treatment. Implantable insulin pumps with an intraperitoneal insulin administration route mimic the physiological route of insulin and should not lead to the peripheral hyperinsulinemia and hepatic hypoinsulinemia that the subcutaneous route does. Studies evaluating the modification of the lipid profile after the replacement of subcutaneous with intraperitoneal insulin treatment have, however, yielded conflicting results. The HDL cholesterol concentrations have been shown to be decreased (226) or unchanged (227-229), the triglycerides increased (226) or unchanged (227-229), and the total cholesterol and ApoB unchanged (227, 228). Even though the subcutaneous route of insulin administration occasionally seems to be associated with more favorable quantitative changes in the lipid profile, it could, however, also be associated with unfavorable qualitative changes, which could affect the function of the lipoprotein particles.

2.3.2 Insulin resistance and lipoprotein metabolism

Insulin resistance is a typical feature of the metabolic syndrome and type 2 diabetes, but as the prevalence of overweight and obesity is increasing in the society this feature is becoming more common in patients with type 1 diabetes as well. Intensive glycemic control can also cause overweight and insulin resistance, and these features are especially common in those with a family history of type 2 diabetes. Moreover, renal disease is associated with insulin resistance already at the early stages in patients both with and without diabetes (230, 231). Insulin resistance is also considered to be a strong pathogenic contributor to the progression of renal disease (232, 233).

Because LPL is insulin-dependent, its activity is commonly reduced in patients with insulin resistance (234), resulting in longer residence times of the TRLs in the circulation. The increased amounts of VLDL particles result, in turn, in an increased triglyceride content in LDL particles through the action of the cholesteryl ester transfer protein (CETP) (235). Triglyceride-rich LDL particles are good substrates for HL, which hydrolyzes triglycerides, making the LDL particles smaller and denser (236). Small, dense LDL (sdLDL) particles are frequently present in insulin-resistant states (237). In children with type 1 diabetes, as many as 87% had a phenotype dominated by the presence of sdLDL compared with only 11% in children without diabetes (238). SdLDL particles have been found to be more atherogenic than the large, buoyant LDL particles for several reasons: i) hepatic LDL receptors have a lower affinity for sdLDL particles, which leads to

a prolonged plasma retention time of these particles (239), ii) sdLDL particles show increased binding to intimal proteoglycans, which could favor penetration into the arterial wall (240), iii) they are more effective in promoting lipid accumulation in macrophages, which leads to an increased formation of foam cells (241), iv) they are more likely to undergo glycation (242), and v) they are more susceptible to oxidation (243, 244). Oxidized LDL (oxLDL) particles are rapidly taken up by macrophages and promote the formation of cytokines (e.g. tumor necrosis factor alpha [TNF-α] and IL-6) by macrophages, accelerating the inflammatory atherosclerotic process (245). In insulin-resistant states, the LDL cholesterol concentrations are only slightly increased or similar to those of controls, but the number of LDL particles is increased (246) (see Figure 6 for illustration). Thus, as 90% of the circulating ApoB is found in LDL, ApoB is consequently also increased when insulin resistance is present and can be seen as a surrogate marker for the number of LDL particles (246).

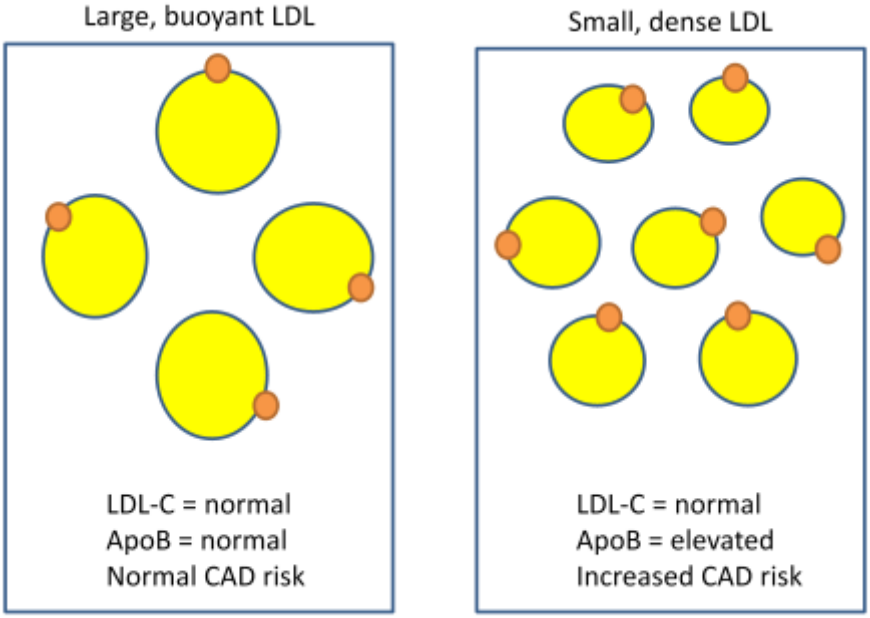


Figure 6. Illustration of the same LDL cholesterol concentrations with either normal or elevated apolipoprotein (Apo) B concentrations.

The main reason for the decreased HDL cholesterol concentrations in insulin resistance seems to be the increased transfer of triglycerides from the TRLs to the HDL particles and the reciprocal transfer of cholesterol from the HDL particles to the TRLs via the CETP enzyme (235). Triglyceride-rich HDL particles are also good substrates for HL, making them smaller and denser, and this increases the catabolism and clearance of HDL particles from the plasma (247) .The reduced HDL concentrations in insulin-resistant states are typically seen as reduced HDL_{2b} subspecies and an increase in the smaller and denser HDL_{3b} and HDL_{3c} subspecies (248). Mechanisms affecting HDL particles in insulin resistance are shown in Figure 7.

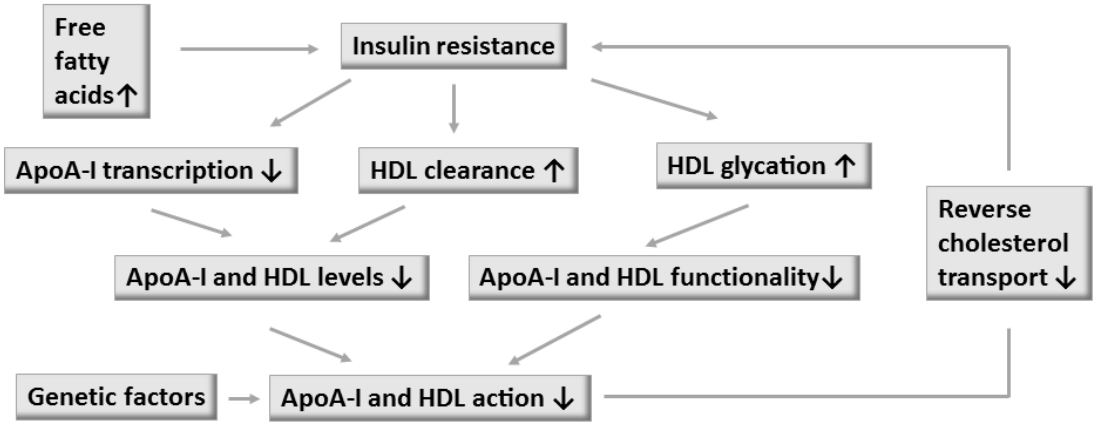


Figure 7. Mechanisms affecting HDL cholesterol and apolipoprotein (Apo) A-I concentrations and functionality in insulin-resistant states (modified from Drew BG et al., Nature Reviews Endocrinology 2012) (249).

2.3.3 Apolipoproteins in diabetes

Insulin exerts multiple effects on the major apolipoproteins (ApoB, ApoA-I, ApoC-III, and ApoE). In patients with type 1 diabetes and fairly good glycemic control, the ApoB concentrations are within the normal range (224) and the ApoA-I concentrations are slightly elevated or within the normal range (250). In normal physiology, insulin promotes ApoB degradation in the hepatocyte and leads to decreased ApoB secretion from the liver (215). In type 1 diabetic patients with ketoacidosis and total lack of insulin, the ApoB concentrations are in the upper normal range, but decrease during insulin treatment with a concordant and significant decrease in VLDL ApoB, but not in IDL or LDL ApoB (220). In insulin-resistant states, the ApoB degradation is decreased, there is an increased production of large $VLDL_1$ particles, and the catabolic rate of ApoB-containing lipoproteins, especially IDL and LDL, is reduced (215, 251). Interestingly, increased ApoB concentrations can already be observed in children with type 1 diabetes as well as in healthy children with diabetic parents compared with healthy children with non-diabetic parents (252). The ApoA-I concentrations are near the normal range already before the initiation of insulin treatment in type 1 diabetic patients with ketoacidosis; however, the ratio of ApoA-I to cholesterol in the HDL particles falls during treatment (220). In insulin-resistant states, the triglyceride loading of core HDL leads to rapid triglyceride lipolysis and the formation of denser HDL particles, and the loss of HDL core triglycerides, in turn, leads to the release of ApoA-I from HDL particles. ApoA-I then undergoes glomerular filtration and the catabolic loss of ApoA-I is increased by 48% (251, 253). However, ApoA-I production is increased by 25%, probably due to a compensatory mechanism, but the net effect is still a reduction in ApoA-I concentrations.

In insulin-resistant states, the inhibitory role of insulin in ApoC-III expression may be lost and the high glucose levels may further stimulate ApoC-III expression (254). Increased free fatty acid delivery to the liver also increases the ApoC-III secretion. ApoC-III inhibits the LPL-mediated catabolism of VLDL and the uptake of VLDL by the liver and may also increase VLDL secretion (203). The ApoE polymorphism influences total and LDL cholesterol as well as ApoB concentrations, but the allele frequency of ApoE in patients with type 1 diabetes does not differ from that of the general population in Finland (218). Glycation is also likely to affect the function of apolipoproteins, and in patients with hyperglycemia glycation of ApoA-I, ApoA-II, ApoB, ApoC-I, and ApoE has been observed (255).

2.3.4 Secondary changes in lipid profile in nephrotic syndrome

Nephrotic syndrome is defined as proteinuria of >3.5 grams/24 h/1.73 m^2 (256). It is accompanied by hypoalbuminemia, edema, thrombophilia, increased risk of infections, and dyslipidemia. The low plasma albumin concentrations, the oncotic pressure, and the renal protein leakage are thought to play important roles in the development of lipid abnormalities. Impaired catabolism and increased synthesis of ApoB-containing lipoproteins and their remnants as well as an accumulation of oxLDL particles is observed in patients with the nephrotic syndrome (257, 258). Lp(a) has also been found to be elevated in patients with the nephrotic syndrome (259), but this can be reversed by antiproteinuric treatment (260). The plasma concentrations of total HDL cholesterol are often normal in patients with nephrotic syndrome, but the maturation of the HDL particles is impaired and qualitative alterations can therefore be seen. Urinary loss of LCAT results in plasma LCAT deficiency and as a consequence the maturation of the small, dense HDL_3 particles to large, buoyant HDL_2 particles is altered (261). This leads to high concentrations of HDL_3 and low concentrations of HDL_2 particles and may also cause disturbances in the reverse cholesterol transport system.

2.3.5 Possible mechanisms for lipid-induced renal injury

The mechanisms by which dyslipidemia could cause or induce renal injury remains unclear, but similarities between atherosclerosis and glomerulosclerosis were recognized already more than 20 years ago (262). Notably, genetic lipid disorders, such as deficiency in LCAT, abnormalities in ApoE, and familial type III hyperlipoproteinemia, lead to renal disease (263-265). Further, when guinea-pigs and rats are fed cholesterol-rich food, they develop various forms of glomerular and other injuries (266, 267). On the other hand, lipid abnormalities alone may be insufficient to cause renal injury since dyslipidemia in non-diabetic human individuals is rarely associated with renal disease. Therefore, there is likely a trigger that causes the initial renal injury, which is then aggregated by dyslipidemia. Hyperglycemia, hypertension, or inflammation, often seen in patients with diabetes, could serve as such a trigger (see also Figure 1).

Lipoproteins enhance matrix expansion, mesangial cell proliferation, and mesangial cytokine production (268-270). The increased cytokine production may recruit macrophages, and similarly as observed in the arterial wall, infiltration of macrophages and foam cells can be found in the glomeruli of patients with diabetic nephropathy (271). Hyperglycemia causes mitochondrial overproduction of reactive oxygen species (ROS) and synthesis of AGEs (272). Further, glycated VLDL and LDL particles are more susceptible to oxidation (273), and AGE-modified LDL particles are cleared from the plasma more slowly (274). As the kidney is a major site for AGE adduct clearance, it is thought that AGE-modified lipoproteins may damage the glomeruli. Also, hyperlipidemia on its own can promote inflammation and the generation of ROS by monocytes (275). Both monocytes and mesangial cells may oxidize lipoproteins (275, 276), and oxLDL particles may serve as chemoattractants for both T-lymphocytes and macrophages (277). Scavenger receptors on the mesangial cells have higher affinity for oxLDL than for native LDL, and stimulation by oxidized lipoproteins leads to mesangial cytokine production and further recruitment of monocytes (274). Cytokine production by tubular epithelial cells is stimulated by the presence of cytokines and high molecular proteins in the glomerular filtrate. Further, oxLDL particles may increase apoptosis of mesangial cells, endothelial cells, and podocytes (278-280). OxLDL can also cause vasoconstriction by increasing the production of vasoactive substances and reducing the production of vasodilators (281). Inflammatory factors, such as TNF-α, ROS, and oxLDL, may cause disruption of the glycocalyx, which is vasoprotective and influences the glomerular permeability (282). OxLDL, hyperglycemia, and ROS can also stimulate the production and activity of the transforming growth factor (TGF)-β (274, 283). TGF-β activation leads to an increase in the synthesis of extracellular matrix proteins and an impairment in extracellular matrix degradation (284). Moreover, TGF-β stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (Nox2), and Nox4 expression in kidney fibroblasts, activating the extracellular signal-regulated kinase ½ (ERK½) pathway. This results in conversion of fibroblasts to a myofibroblast phenotype, which is associated with interstitial fibrosis (285). Dyslipidemia may also cause alterations in the coagulation-fibrinolysis system, a decreased renal blood flow, and endothelial cell damage (286).

2.4 Lipid-lowering treatment

Statins inhibit the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme, which plays a central role in hepatic cholesterol synthesis (287). Strong evidence indicates that statins reduce CVD events in patients with diabetes, but almost all trials in patients with diabetes have mainly included patients with type 2 diabetes. The trials including the largest numbers of patients with diabetes are the Heart Protection Study (HPS) (288), the Anglo-Scandinavian Cardiac Outcomes Trial – Lipid-Lowering Arm (ASCOT-LLA) (289), the Collaborative Atorvastatin Diabetes Study (CARDS) (290), and the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial – Lipid-Lowering Trial (ALLHAT-LLT) (291) including almost 15 000 patients with

diabetes. The largest cohort of patients with type 1 diabetes is found in the HPS with 615 patients (288). In this particular trial, the magnitude of the reduction in CVD events was similar in patients with type 1 and type 2 diabetes, although the reduction was not statistically significant in the former group due to lower power. In a meta-analysis of 14 trials including 18 686 patients with diabetes (1466 with type 1 diabetes), major vascular events were reduced by 21% per 1 mmol/l reduction in LDL cholesterol during an average follow-up of 4.3 years (292). The findings were independent of the baseline lipoprotein concentrations, and similar benefits were seen irrespective of age, sex, type of diabetes, or kidney status. Based on this meta-analysis, the number needed to treat (NNT) to prevent one vascular event was as low as 9 for high-potency statins and 22 for low-potency statins. Economic analyses of randomized statin trials, including the HPS, have shown that statin treatment is cost-effective for a wide range of patients with diabetes (293).

The lipid-lowering mechanisms of the fibrates, i.e. peroxisome proliferation activator receptor (PPAR)-α agonists, include activation of LPL and reduced production of ApoC-III, leading to an increased clearance of VLDL and IDL particles (294). Rather inconsistent results have been reported on the effect of fibrates on cardiovascular outcomes. In a meta-analysis with 45 058 participants, a 13% relative risk (RR) reduction was found for CAD events, but no benefits on stroke or all-cause mortality were seen. In patients with type 2 diabetes, the results regarding the overall primary end-points have been disappointing, but beneficial effects on CVD risk in patients with dyslipidemia (defined as high triglycerides and low HDL cholesterol) at baseline have consistently been reported (295-297). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study, a significant 27% RR reduction was observed in patients with marked dyslipidemia (triglycerides ≥2.3 mmol/l alone or with low HDL cholesterol concentrations) at baseline treated with fenofibrate for 5 years compared with the non-significant 11% RR reduction in the entire cohort (296).

2.4.1 Lipid-lowering treatment and progression of renal disease

Randomized clinical trials of lipid-lowering treatment with data on renal disease progression are listed in Table 2.

Treatment with pravastatin or simvastatin has been associated with a modest reduction in the rate of eGFR decline in non-diabetic cohorts with (or with a high risk of) coronary heart disease (298, 299), and instead of the expected 5-year decline in eGFR, an improvement of eGFR was seen in patients with atorvastatin treatment (300). In the HPS trial, including patients with diabetes or occlusive arterial disease, simvastatin treatment was associated with a smaller decrease in eGFR, and the effect was slightly larger among patients with diabetes (288). In the CARDS Trial, comprising patients with type 2 diabetes without prior CVD, atorvastatin treatment was associated with a modest improvement in the annual change in eGFR (301), and in a substudy of the Treating to New Targets (TNT) trial in patients with diabetes and CAD, 10 mg and 80 mg of atorvastatin increased eGFR

in patients with or without moderate CKD, with a more significant increase in eGFR in patients treated with the higher atorvastatin dose (302). In a meta-analysis of 39 704 patients, including both patients with and without diabetes, the eGFR decline was 1.22 ml/min/year slower with statin treatment; however, subgroup analyses showed no significant differences in patients with diabetic nephropathy and the between-study heterogeneity was considerable (303). In a recently published meta-analysis of 41 trials with a total of 88 523 patients, statin treatment modestly reduced the rate of decline in eGFR compared with placebo (standardized mean difference 0.15, p=0.0004) (304). Twenty-one of these trials with a total of 3933 patients reported data on urinary protein excretion, and a modest decrease in proteinuria with statin treatment was also seen. High- and moderate-intensity statins significantly decreased the rate of reduction of eGFR, whereas the difference between low-intensity statins and placebo was non-significant. The authors concluded that the beneficial effect of statin treatment might be dosage-related and duration-dependent. Statins reduced the decline in eGFR in patients with stage 1 to 3 CKD, but data were insufficient to analyze the effect of statins on patients with stage 4 to 5 CKD.

In the Justification for the Use of Statins in Prevention - an Intervention Trial Evaluating Rosuvastatin (JUPITER), no effect on eGFR was seen after 12 months of rosuvastatin treatment (305), and a combination therapy of simvastatin and ezemitibe (an inhibitor of cholesterol absorption in the gut) did not affect measures of kidney disease in pre-dialysis patients in the Study of Heart and Renal Protection (SHARP) Trial (306). Further, fluvastatin treatment had no effect on the incidence of renal graft loss, doubling of serum creatinine, or decline in GFR during a 5-year follow-up of 2102 renal transplant recipients in the Assessment of Lescol in Renal Transplantation (ALERT) Study (307, 308). However, in a meta-analysis including nine trials with atorvastatin treatment for 4194 patients with pre-dialysis CKD, a significant effect on eGFR was reported (309).

Fenofibrate treatment is associated with an initial increase in plasma creatinine, and hence, a reduction of eGFR, but the increase has been reported to be reversible (310, 311), and in a small study a reduction of GFR assessed by inulin clearance was not observed (312). The results from the FIELD Study have been conflicting. In the FIELD Helsinki Substudy, a decrease in eGFR and an increase in cystatin C with fenofibrate treatment were reported (313). In another substudy of the FIELD cohort, fenofibrate initially decreased eGFR, but after a washout period, eGFR had fallen less from baseline with fenofibrate than with placebo treatment, 1.9 vs. 6.9 ml/min/1.73 m^2 ($p<0.001$, after ~5 years), and a greater benefit of eGFR preservation was seen with fenofibrate treatment in those with baseline dyslipidemia (i.e. high triglycerides and low HDL cholesterol) (310). In the Diabetes Atherosclerosis Intervention Study (DAIS), fenofibrate treatment reduced progression to microalbuminuria, but the mean values of AER did not change (314). In the main results of the FIELD Study, fenofibrate treatment modestly reduced the pooled end-point of progression or regression of albuminuria status (295); however, in the Helsinki Substudy, no beneficial effect on AER analyzed as a continuous variable could be seen (313). The results of the FIELD Study may have been weakened by the use of other lipid-lowering

treatment (mainly statins) in 36% of patients in the placebo group and in 19% of patients in the fenofibrate group by the end of the study (295). However, in the ACCORD Study, all patients received simvastatin treatment, but on top of this patients were randomized to receive either fenofibrate or placebo (297). The combination of fenofibrate and simvastatin modestly reduced progression to micro- or macroalbuminuria compared with simvastatin treatment alone. In a meta-analysis with 14 385 patients with type 2 diabetes, fenofibrate reduced the risk of albuminuria progression by 14% (315). Data on regression of albuminuria status were available for 2152 patients, and the likelihood of regression increased (RR 1.19) with fenofibrate treatment.

All in all, lipid-lowering treatment seems to have a modest beneficial effect on the decline of eGFR and the development and progression of albuminuria. Most of the current evidence on lipid-lowering treatment comes from patients with vascular disease already present at baseline, and it is not known whether lipid-lowering intervention at an earlier stage could provide benefits that may be lost at the later stages of diabetic nephropathy. Further, the follow-up times of most randomized clinical trials are fairly short, and larger benefits would likely be observed with longer treatment periods initiated earlier in the course of the disease. For example, a loss-of-function mutation leading to a lifelong reduction of LDL cholesterol of ~1.0 mmol/l was associated with ~88% reduction of CAD (316), whereas LDL cholesterol lowering of a similar magnitude with statin treatment for 5 years reduces CAD events by ~35% (317). Animal studies have also suggested that the combined effect of ACE inhibitors and statins might provide larger renal benefits than either drug alone (318). In the future, the Adolescent type 1 Diabetes, cardio-renal Intervention Trial (AdDIT) will show the results of the combined effect of an ACE inhibitor (quinapril) and atorvastatin on early surrogate measurements of diabetic nephropathy and CVD (319) and hopefully provide us with new insight. Furthermore, trials with hard renal end-points and direct GFR measurements (not only eGFR, which is dependent on creatinine production and excretion) are also needed to clarify the situation.

Table 2. Studies of lipid-lowering treatment and progression of renal disease.

Study (ref)	Intervention	Patients	N, follow-up time	Renal outcome	Additional information
ACCORD (297)	Fenofibrate 160 mg/PBO	T2DM, high vascular risk, all patients received 20-40 mg simvastatin	N=5518, 4.7 years	Incidence of micro 38.2% vs. 41.6% (p=0.01). Incidence of macro 10.5% vs. 12.3% (p=0.04)	Reduced dose of fenofibrate if eGFR <50 ml/min/1.73 m^2
DAIS (314)	Fenofibrate 200 mg/PBO	T2DM without nephropathy	N=314, 3.3 years	Reduction of progression to micro 3.0% vs. 17.7% (p<0.001)	8% on fenofibrate and 18% on PBO had higher AER at trial end
FIELD (295)	Fenofibrate 200 mg/PBO	T2DM, majority without overt nephropathy	N=9795, 5 years	2.6% less progression or more regression of albuminuria (p=0.002)	Statistically significant if pooled with regression of albuminuria
HPS (288)	Simvastatin 40 mg/PBO	T1DM (3%), T2D(26%), arterial disease without DM (71%)	N=20 270, (5963 with DM) 4.6 years	Slower decrease in eGFR 5.9 vs. 6.7 ml/min/1.73 m^2 (p=0·0003) during follow-up	Effect on eGFR larger in patients with diabetes
CARDS (301)	Atorvastatin 10 mg/PBO	T2DM, no prior CVD, 34% impaired eGFR	N=2838, 3.9 years	Net improvement in eGFR 0.18 ml/min/1.73 m^2/year	Net improvement 0.38 ml/min/1.73m^2/year in those with albuminuria
TNT (302)	Atorvastatin 10 or 80 mg	T2DM, CAD	N=1431, 4.8 years	Improvement in eGFR at the end of follow-up in both treatment groups 0.5 vs. 2.6 ml/min/1.73 m^2 (p=0.001)	
ALERT (307, 308)	Fluvastatin 40 mg/80 mg/PBO	Renal transplant recipients, 13% with DM	N=2102, 6 years	No effect	GFR measured directly in a subset of 439 patients
SHARP (306)	Simvastatin 20 mg and Ezetimibe 10 mg/PBO	Dialysis or pre-dialysis patients, 20% with DM	N=9270, 4.9 years	No effect	Simvastatin 20 mg alone in 1054 patients for one year

PBO = placebo, T1DM = type 1 diabetes mellitus, T2DM = type 2 diabetes mellitus, micro = microalbuminuria, macro = macroalbuminuria

2.4.2 Lipid-lowering treatment and progression of diabetic retinopathy

Data on the effect of statin treatment on diabetic retinopathy are lacking from large randomized clinical trials, but because statin treatment is such an important part of the prevention of CVD, it would today be considered unethical to have a placebo arm in a trial including patients with increased CVD risk at baseline. Previously, the CARDS showed a trend towards a reduced need of retinal laser treatment with atorvastatin treatment, but no impact on the progression of diabetic retinopathy was seen (290). In contrast, large trials investigating the effect of fibrate therapy on the development and progression of diabetic retinopathy have been performed. In the FIELD Study, fenofibrate treatment significantly reduced the need for the first laser treatment due to either PDR or macular edema (hazard ratio [HR] 0.69). However, the need for laser treatment was not predicted by the baseline plasma lipid concentrations (150). The NNT for prevention of laser treatment in patients with pre-existing retinopathy was fairly low and clinically worthwhile (NNT=17), while the results for primary prevention of diabetic retinopathy were not as convincing (NNT=90). In the FIELD Ophthalmology Substudy, a 2-step progression on the ETDRS severity scale did not differ between the two groups overall, but among patients with pre-existing retinopathy, fewer patients on fenofibrate had a 2-step progression than in the placebo group. In the ACCORD Study, fenofibrate together with simvastatin treatment reduced the rate of progression of diabetic retinopathy, defined as at least a 3-step progression on the ETDRS scale or the development of PDR (HR 0.60) compared with simvastatin treatment alone (167).

2.4.3 Lipid-lowering treatment and risk of development of type 2 diabetes

Cardiovascular risk factors and risk factors for type 2 diabetes are often present in the same patients, therefore, many patients who are prescribed statin treatment already have an increased risk for development of type 2 diabetes before initiation of statin treatment. However, recent data have shown that statin treatment in itself is associated with a modestly increased risk of type 2 diabetes. In a meta-analysis, statin treatment increased the risk of diabetes development by 9% (320). In another meta-analysis, intensive-dose treatment was associated with a 12% increased risk compared with moderate-dose statin treatment, but intensive-dose treatment was also associated with fewer major CVD events (OR 0.84) (31). Simvastatin, atorvastatin and rosuvastatin have all been associated with an increased diabetes risk, but the results regarding pravastatin treatment have been conflicting, and even protective effects have been reported (321). In an analysis from the Jupiter Trial, rosuvastatin treatment was associated with a 39% reduction of the primary CVD endpoint and a 28% increase in diabetes in patients with common risk factors for diabetes, but in patients without risk factors for diabetes no increase in the risk of diabetes development was seen (322). The increased diabetes risk is observed especially in older patient groups. In a Finnish study, less weight loss during statin treatment was observed in

elderly men (median age 73 years), hence, the possible positive protective effect of statin treatment against frailty may paradoxically lead to a higher diabetes risk (323). Further, a Mendelian randomization study showed that genetic variants in the *HMG-CoA* gene associated with lower LDL cholesterol were also associated with an increased risk of type 2 diabetes and higher bodyweight (324). All in all, the risk of diabetes development with statin treatment is fairly low and the cardiovascular benefits outweigh the diabetogenic risk. The guidelines regarding cardiovascular prevention and statin treatment have therefore not been altered. However, patients receiving statin treatment who have risk factors for diabetes should be informed about the risk, receive support for lifestyle changes, and regularly be monitored for hyperglycemia.

3 AIMS OF THE STUDY

The main aims of this thesis were as follows:

I To examine the relationship between lipid variables, eGFR, and AER. A further aim was to assess the effect of glycemic control, obesity, and hypertension on lipid profiles in patients without renal disease.

II To evaluate the impact of baseline lipid values on the progression of renal disease in patients with type 1 diabetes at all stages of albuminuria.

III To investigate the association between lipid variables and diabetic retinopathy in patients with type 1 diabetes. Furthermore, interactions and correlations between diabetic retinopathy, nephropathy, and lipid variables were explored.

IV To assess the ability of lipid variables to predict incident CAD events in patients with type 1 diabetes. Moreover, the effect of renal disease, sex, and glycemic control on the ability of the lipid variables to predict CAD events was explored.

4 SUBJECTS AND STUDY DESIGN

4.1 The FinnDiane Study

These studies are part of the ongoing prospective Finnish Diabetic Nephropathy Study (FinnDiane), a nationwide, multicenter study, initiated in 1997 that recruited its first patients in January 1998. Prior to the FinnDiane Study, two pilot studies, GENREL and NEFREL, that recruited families with diabetic nephropathy existed and the patients from these studies were also included in the FinnDiane population. Follow-up data have been collected since 2004. The aim of FinnDiane is to identify genetic, environmental, and clinical risk factors for micro- and macrovascular complications in patients with type 1 diabetes, with a special emphasis on diabetic nephropathy. The main aims of FinnDiane are to cover ~25% of all adult patients with type 1 diabetes in Finland and to answer the question: why do one-third of patients with type 1 diabetes develop diabetic nephropathy? Adult patients from 77 hospitals and primary healthcare centers in Finland have consecutively been asked to participate in the study, and the response rate has been about 78% (325). The study centers include all 5 university central hospitals, all 16 central hospitals, 26 other hospitals, and 30 primary healthcare units in Finland (listed in the Appendix). The geographic distribution of the FinnDiane patients (Figure 8) closely follows the distribution of the general population in Finland, and the high response rate makes any major significant biases unlikely. At the moment around 5000 patients with type 1 diabetes have been recruited, which represents about 12.5% of all patients with type 1 diabetes in Finland. In each study of this thesis, we started with the inclusion of all patients with centrally measured lipid profiles available from the database at the time of the study and then added the selection criteria for each study, which are explained in more detail below.

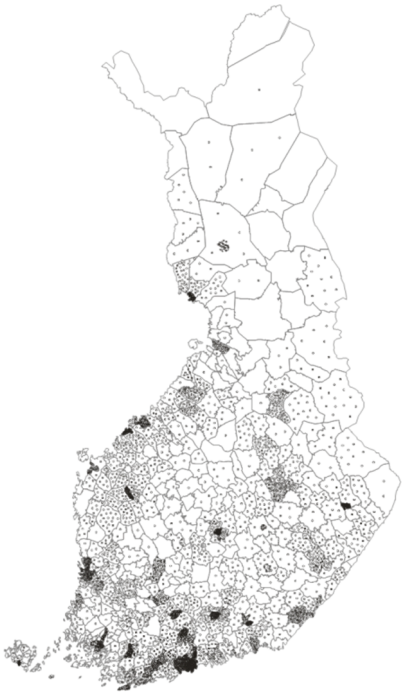


Figure 8. Distribution of the FinnDiane patients. Each dot indicates one FinnDiane patient. The distribution is similar to the distribution of the general population in Finland.

4.2 Collection of cross-sectional data

At baseline, patients underwent a thorough clinical investigation at a regular visit to their attending physician. Data on diabetes duration, medication, diabetic microvascular complications, smoking, and cardiovascular disease were registered based on medical records and obtained by the patient's attending physician using a standardized questionnaire. The baseline data were obtained from patients who participated in the FinnDiane Study between 1994 and 2008.

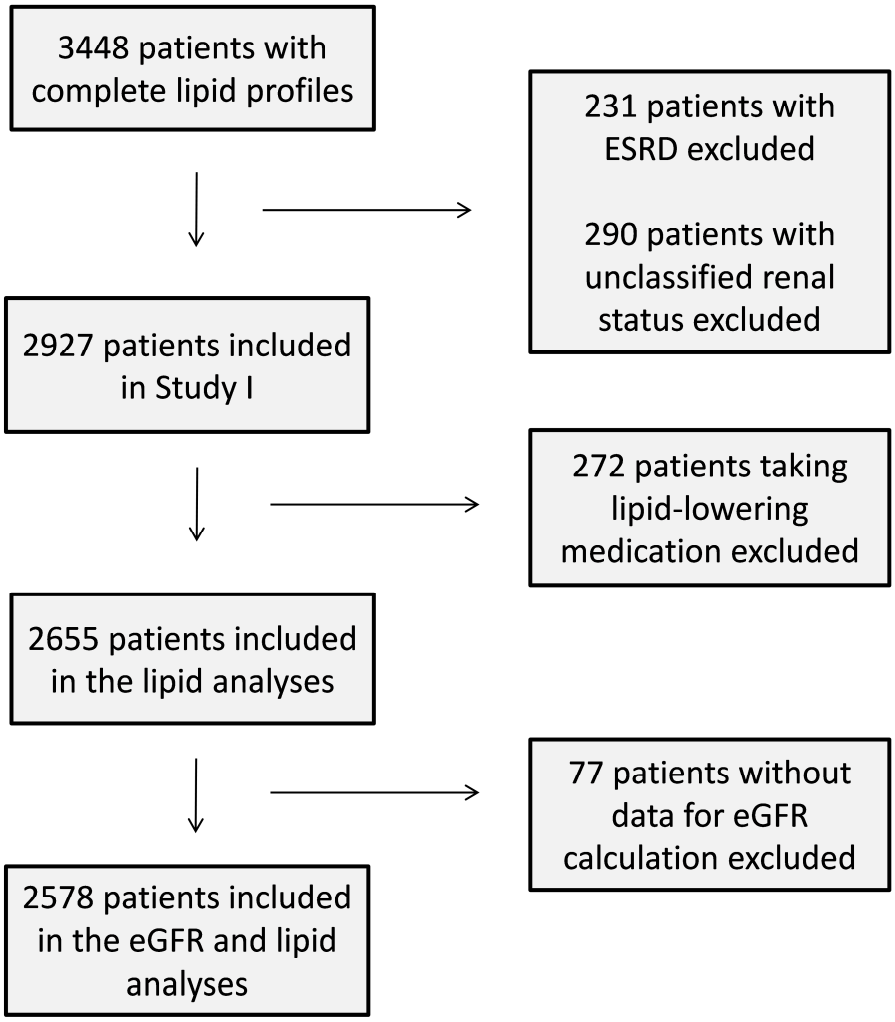


Figure 9. Flow chart of patient selection in Study I.

Study I: At the time of the study, complete lipid profiles were obtained from 3448 patients; the selection criteria are described in Figure 9. The clinical characteristics of the patients grouped by albuminuria status can be seen in Table 3.

Table 3. Clinical characteristics of subjects grouped by albuminuria status.

	Normal AER	Micro	Macro
N	1959	453	515
Men (%)	48.2	58.5	57.9
Age (years)	35.5 ± 11.9	37.9 ± 11.7	40.9 ± 9.8
Age at onset (years)	16.2 ± 8.5	11.8 ± 7.9	11.7 ± 7.2
Diabetes duration (years)	19.3 ± 11.6	26.1 ± 10.7	29.2 ± 7.8
Systolic blood pressure (mmHg)	129 ± 15	135 ± 17	144 ± 19
Diastolic blood pressure (mmHg)	78 ± 9	80 ± 10	83 ± 10
Body mass index (kg/m^2)	24.8 ± 3.3	25.6 ± 3.6	25.8 ± 4.0
HbA_{1C} (%)	8.2 ± 1.4	8.8 ± 1.5	9.0 ± 1.5
Albumin excretion rate (mg/24h)	8 (5-13)	56 (26-107)	497 (166-1270)
Serum creatinine (μmol/l)	83 ± 16	90 ± 19	171 ± 125
Current smoking (%)	22.2	29.6	29.1

Data are means ± SD, median (IQR) or %. AER = albumin excretion rate, Micro = microalbuminuria, Macro = macroalbuminuria.

Study III: Retinopathy status was determined in 1465 consecutively recruited patients with complete lipid profiles. To avoid selection bias, these patients represent the first consecutive patients participating in the FinnDiane Study. In addition, an independent data set of 1110 patients without ESRD and a minimum diabetes duration of 10 years was evaluated to replicate the interaction and correlation analyses.

4.3 Collection of follow-up data

Study II: At follow-up, all available medical files, including laboratory data, were reviewed and any changes in renal status were verified. Prospective data were available for 2412 patients. Patients with ESRD (n = 143) were excluded. Altogether, 2304 patients participated in the study and were followed for 5.4 ± 2.0 years. Progression was defined as a change from a lower to a higher level of albuminuria (normal AER to microalbuminuria, or micro- to macroalbuminuria) or development of ESRD in patients with macroalbuminuria at baseline.

Study IV: Complete baseline data, including centrally measured lipid profiles, were available for 3872 patients. Follow-up data were obtained from the Finnish Hospital Discharge Register (HILMO) based on hospital discharge records and the Causes of Death Register through to 31.12.2010 and available for all patients. Patients with acute myocardial infarction, coronary artery bypass graft surgery, or coronary angioplasty at baseline were excluded (N=306). Further, patients with International Classification of

Diseases (ICD)-10 diagnosis codes I20 and I22-25 (ICD-9: 411-414) in the Finnish Hospital Discharge Register, those with reported coronary heart disease, or those taking long-acting nitroglycerin medication at baseline were excluded from the patient group without an event during the follow-up period (N=46). Hence, a total of 3520 patients were included and followed for a median of 10.2 (8.6-12.0) years.

4.4 Ethical aspects

The FinnDiane Study protocol was approved by the Ethics Committee of Helsinki University Central Hospital (decision number: 491/E5/2006) as well as by the local ethics committees of each participating study center and is being conducted in accordance with the Declaration of Helsinki. All patients gave their written informed consent before participation in the study. All research files used for data analyses were coded with ID numbers and personal information is known only to the FinnDiane researchers.

The FinnDiane Study is an observational study; hence, no interventions for patients are carried out. The only potential nuisance to patients is the possible pain caused by venapuncture when blood samples are drawn as well as the extra time spent during study visits and in completing questionnaires.

5 METHODS

5.1 FinnDiane Study protocol

5.1.1 Definition of type 1 diabetes

Type 1 diabetes was defined as onset of diabetes before the age of 35 years and with permanent insulin treatment initiated within one year of diagnosis. Adult patients with type 1 diabetes from 77 hospitals and primary healthcare centers all over Finland were consecutively invited to participate.

5.1.2 Definition of diabetic nephropathy

Nephropathy status was determined based on the measurement of albumin excretion rate (AER) in at least two of the three consecutive 24-h or overnight urine collections.

Normal AER: AER<20 µg/min or <30 mg/24 h	Microalbuminuria: 20≤AER<200 µg/min or 30≤AER<300 mg/24 h	Macroalbuminuria: AER≥200 µg/min or AER≥300 mg/24 h	ESRD: Dialysis or kidney transplant

5.1.3 Assessment of renal function

Estimated glomerular filtration rate (eGFR) was calculated on the basis of a single serum creatinine measurement using the Cockcroft–Gault formula adjusted for body surface area (71) (Studies I and II), the Modification of Diet in Renal Disease (MDRD-4) equation (72) (Studies II and III), or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (73) (Study IV).

The Cockcroft-Gault formula is calculated as:

$$eGFR = ([140-age] \times weight\ [kg] \times constant) / creatinine$$

-the constant is 1.23 for men and 1.04 for women

The MDRD-4 equation is calculated as:

$$eGFR = 32788 \times creatinine^{-1.154} \times age^{-0.203} \times (0.724\ if\ female)$$

The CKD-EPI formula is calculated as:

a) Female S-Creatinine ≤ 61.9 µmol/l: $eGFR = 144 \times (creatinine / 61.9)^{-0.329} \times (0.993)^{age}$
b) Female S-Creatinine > 61.9 µmol/l: $eGFR = 144 \times (creatinine / 61.9)^{-1.209} \times (0.993)^{age}$
c) Male S-Creatinine ≤ 79.6 µmol/l: $eGFR = 141 \times (creatinine / 79.6)^{-0.411} \times (0.993)^{age}$
d) Male S-Creatinine > 79.6 µmol/l: $eGFR = 141 \times (creatinine / 79.6)^{-1.209} \times (0.993)^{age}$

In Study I, patients were divided into three groups on the basis of the eGFR calculated with the Cockcroft–Gault formula:

Normal renal function: >90 ml/min/1.73 m^2	Mildly impaired renal function: 60-90 ml/min/1.73 m^2	Impaired renal function: <60 ml/min/1.73 m^2

5.1.4 Definition of diabetic retinopathy

Fundus photographs taken by the participating study centers were scanned and stored in a digital archive; these were available for 1128 (77%) of 1465 patients. Ophthalmic records with information about fundus examinations were also obtained. The clinical fundus examination is important because it has good specificity (326) and the combination of fundus photographs and a clinical examination provide both good sensitivity and specificity for detection of severe diabetic retinopathy. The data were analyzed by a specialist in ophthalmology (Kustaa Hietala) who was unaware of the demographic data and the presence of other complications. The ETDRS scale was used to grade the severity of diabetic retinopathy, with 10 defined as no diabetic retinopathy, 20-35 as mild non-proliferative diabetic retinopathy (NPDR), 43-53 as moderate to severe NPDR, and 61 and over as proliferative diabetic retinopathy (PDR) (327). The eye with the more severe diabetic retinopathy was used to determine the retinopathy stage for a patient. For patients without available fundus photographs the verbal descriptions of clinical fundus examinations by ophthalmologists were transformed to approximate numerical values on the ETDRS scale. In an independent cohort in Study III, severe diabetic retinopathy was defined as history of laser photocoagulation. The underlying cause for laser treatment was PDR in the majority (>80%) of patients, and the rest of the patients received laser photocoagulation mainly due to macular edema or severe non-proliferative retinopathy (161).

ETDRS severity scale:

No retinopathy: Level 10	Mild NPDR: Level 20-35	Moderate to severe NPDR: Level 43-53	PDR: Level ≥ 61

5.1.5 Definition of cardiovascular disease (CVD)

A CVD event was defined as a history of myocardial infarction, stroke (cerebral infarction or intracerebral hemorrhage), or amputation. In Study I, coronary heart disease (CHD) was defined as myocardial infarction, coronary revascularization, or regular use of long-acting nitroglycerin.

5.1.6 Definition of coronary artery disease (CAD)

In Study IV, an incident CAD event was defined as myocardial infarction given as ICD-10 code I21 (ICD-9: 410), coronary artery bypass graft surgery, or coronary angioplasty. Author Nina Tolonen from the FinnDiane Study group verified the Finnish Hospital Discharge Register (HILMO) data by reviewing the hospital records of 28% of the patients. In this sample, no typing errors were found, and only four borderline cases of acute myocardial infarction were identified. Otherwise, all cases were in accordance with the universal definition of myocardial infarction (328) or had undergone either coronary artery bypass graft surgery or coronary angioplasty. Fatal CAD events were identified from a search of the Finnish Causes of Death Register and established when the immediate or underlying cause of death was from CAD, i.e. given as ICD-10: I20-25 (ICD-9: 410-414). Death certificates were also obtained to verify the register data.

5.1.7 Anthropometric measurements

Weight and height were recorded with 0.1 kg and 1 cm accuracy, respectively. Waist circumference was measured halfway between the lowest rib and the iliac crest. Hip circumference was measured at the major trochanters of the femurs. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference.
Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

In Study I, patients were divided into three groups based on their BMI:

Normal BMI:	Overweight:	Obesity:
< 25 kg/m^2	25–30 kg/m^2	> 30 kg/m^2

5.1.8 Assessment of blood pressure

Blood pressure was measured twice from the brachial artery at 2-min intervals in the sitting position after a 10-min rest. A manual sphygmomanometer or an automated blood pressure measurement device was used. The mean value of at least two measurements was used in the analyses.

In Study I, hypertension was defined as the use of antihypertensive medication or systolic/diastolic blood pressure higher than 130/80 mmHg (116).

5.1.9 Definition of smoking

Current smoking was defined as smoking at least one cigarette per day at the time of data collection. History of smoking was defined as smoking at least one cigarette per day for a minimum of 3 months but ceasing to smoke before data collection.

5.2 Laboratory measurements and assays

5.2.1 Lipids and lipoproteins

All serum lipid and lipoprotein concentrations were measured from blood samples in Professor Marja-Riitta Taskinen's research laboratory at Helsinki University Central Hospital, Division of Cardiology, Helsinki, Finland. Total cholesterol and triglycerides were determined enzymatically using a Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) with commercially available kits (Hoffman-La Roche until November 2001 and ABX Diagnostics, Montpellier, France until January 2006). Thereafter, an enzymatic determination by Konelab 60i analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and a kit from the same manufacturer were used. Total HDL and HDL_3 cholesterol were determined enzymatically using a HTS 7000 Plus Bio Assay Reader (Perkin Elmer, Waltham, MA, USA) with a commercial kit from Roche Diagnostic Hitachi (Hitachi, Tokyo, Japan). HDL_2 cholesterol was calculated by subtracting HDL_3 cholesterol from total HDL cholesterol. LDL cholesterol was calculated with the Friedewald formula if triglycerides were below 4.0 mmol/l (329). Serum ApoB concentrations were determined using a Cobas Mira analyzer by immunoprecipitation with a commercial kit (Orion Diagnostica, Espoo, Finland) until January 2006. Thereafter, an immunoprecipitation method with a Konelab 60i analyzer and a kit from the same manufacturer (Thermo Fisher Scientific) were used. Serum ApoA-I concentrations were determined with a Cobas Mira analyzer by immunoprecipitation with commercial kits (Boehringer-Mannheim until January 2002 and Wako Chemicals GmbH, Neuss, Germany until January 2006), and thereafter, with a Konelab 60i analyzer by immunoprecipitation (Thermo Fisher Scientific). Serum ApoA-II concentrations were determined with a Cobas Mira analyzer by immunoprecipitation with a commercial kit (Boehringer-Mannheim until August 2001), and thereafter, a polyclonal antibody produced in sheep against human ApoA-II was used.

In Study I, cut-off values based on the recommendation of the American Diabetes Association (116) were as follows: LDL cholesterol ≤2.6 mmol/l, triglycerides ≤1.7 mmol/l, and HDL cholesterol ≥1.0 mmol/l for men and ≥1.3 mmol/l for women.

5.2.2 HbA_{1c}

Glycosylated hemoglobin A_{1c} (HbA_{1c}) was determined locally at each center by standardized assays. In Study I, patients were divided into three groups with regard to glycemic control:

Good glycemic control: HbA_{1c} < 7.5%	Intermediate glycemic control: HbA_{1c} 7.5-9%	Poor glycemic control: HbA_{1c} > 9%

5.2.3 Assessment of insulin sensitivity

To estimate insulin sensitivity, estimated glucose disposal rate (eGDR) was calculated with an equation developed by Williams et al. (124) based on clinical risk factors and validated with euglycemic-hyperinsulinemic clamp measurements in a subset of the Pittsburgh EDC Study population. An equation modified for use with HbA_{1c} instead of HbA_1 was used in this study.

$$eGDR = 24.4 - 12.97 \times WHR - 3.39 \times hypertension - 0.60 \times HbA_{1c}$$

Hypertension is defined as antihypertensive treatment and/or blood pressure ≥140/90 mmHg (yes = 1, no = 0).

5.2.4 Creatinine

Serum creatinine was measured with a kinetic Jaffé reaction using a Hitachi 911 E analyzer (Boehringer Mannheim, Mannheim, Germany) until January 2002. Thereafter, a photometric, enzymatic (isotope dilution mass spectrometry = IDMS) method using a Hitachi 917 or Modular analyzer (Boehringer Mannheim/Roche Diagnostics, Basel, Switzerland) was applied. The correlation coefficient between the two methods is 0.988.

To enable use of the data derived from both methods, the following conversion formula was applied:

$$S\text{-Creatinine (IDMS)} = (0.953 \times S\text{-Creatinine Jaffé}) - 7.261$$

5.2.5 Urinary albumin excretion rate (AER)

In addition to the urine collections used for the classification of renal status, AER was also measured centrally from 24-h urine collections. It was measured by radioimmunoassay using a LKB Wallac RiaGamma counter (Pharmacia, Uppsala, Sweden) until November 2002. Thereafter, an immunoturbidimetric method was used with a Hitachi 911 analyzer (Roche Diagnostics, Hoffman-La Roche, Basel, Switzerland). This measurement was included in the multivariate analyses.

5.3 Lipid-lowering medication

Lipid-lowering medication was defined as the use of statins, fibrates, and/or ezetimibe; however, only a few patients were on fibrate or ezetimibe therapy. The active substance and doses of patients' lipid-lowering medication were registered. In Study I, patients with lipid-lowering medication were excluded from the analyses. In the other studies, parts of the analyses were performed correcting for or excluding patients with lipid-lowering medication.

5.4 Statistical analyses

Data for normally distributed and continuous variables are presented as mean ± standard deviation (SD) and data for non-normally distributed variables as median with interquartile range (IQR). Differences between groups were analyzed with Student´s t-test or ANOVA for normally distributed variables between two or three groups, respectively. Differences between non-normally distributed variables for two groups were analyzed with the Mann-Whitney U-test and for three groups with the Kruskal-Wallis test. Categorical variables were analyzed using Pearson's χ^2 test. Values of p for lipid variables for comparison between groups were adjusted for age, sex, and BMI. Pearson's correlation coefficients were used to calculate correlations between normally distributed values, and Spearman's rank correlation coefficients were used for non-normally distributed values. Non-normally distributed values were logarithmically transformed before inclusion in the multivariate models.

In Study I, multiple linear regression analyses were performed with either eGFR or AER as the dependent variable.

5.4.1 Study II

Cox proportional hazards model was used to investigate the relationship between possible predictors of progression of diabetic nephropathy. Results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). A standard model, including conventional risk

factors, i.e. diabetes duration, HbA_{1c}, SBP, sex, BMI, and current smoking, was used for the analyses. Receiver-operating characteristic (ROC) curves were performed to identify possible thresholds of triglycerides for the prediction of renal disease. The shortest distance on the ROC curve corresponding to the maximum sum of sensitivity and specificity was used in the determination of cut-off points.

5.4.2 Study III

PDR or mild NPDR was the dependent variable in multiple logistic regression analyses. To determine whether the lipid variables have a different effect on AER depending on retinopathy status, interaction terms between retinopathy status (no retinopathy, mild NPDR, moderate to severe NPDR, PDR) and lipid variables were explored with linear regression models where the natural logarithm of (ln)AER was the dependent variable. The relationship between lipid variables and retinopathy status was further analyzed with least square estimates for ln AER, which were calculated after stratification of the data by quartiles of triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and retinopathy status groups. Back-transformation resulted in geometric means adjusted for diabetes duration, SBP, and HbA_{1c} (Figure 1 in Study III). Patients with ESRD and a diabetes duration of less than 10 years were excluded from correlation and interaction analyses and from Figure 1. In the additional correlation and interaction analyses in an independent data set, retinal laser treatment (yes, no) was used to stratify the cohort. Patients with ESRD and diabetes duration of less than 10 years were also excluded from these analyses.

5.4.3 Study IV

To analyze the associations between risk factors and incident CAD events, univariate and multivariate Cox regression models were used. Variables included in the multivariate models were all univariately associated with CAD and every variable reduced the Akaike information criteria (AIC), except for sex, but since it is a well-established CAD risk factor, it was included in the models. The multivariate models included diabetes duration, HbA_{1c}, SBP, sex, WHR, eGFR, retinal laser treatment, AER, history of smoking, and one of the lipid variables. Because of collinearity, only one of the lipid variables was entered into the models at a time. Results are presented as HRs per SD increase, with 95% CIs. The standardized score for WHR was calculated separately for men and women. Fine and Gray regression analyses were also performed to take into account the competing event of non-CAD death (330). After the Fine and Gray competing risks analyses were performed, figures of the cumulative incidence for CAD in normoalbuminuric or macroalbuminuric patients divided by the median of lipid variables were drawn. To compare the ability of the lipid variables to predict an incident CAD event, we calculated the area under the ROC curves (AUC). Further, likelihood ratio (LR) χ^2 statistics from the Cox models were calculated, a higher value indicating a better global fit. Net reclassification improvement

(NRI) is the percentage reclassified after the inclusion of the variable of interest in the above-mentioned multivariate model, distinguishing between movements in the correct direction, i.e. the proportion of subjects being reclassified to a higher risk category amongst CAD cases or a lower risk category amongst controls (331). The 5%, 10%, and 20% cut-off points have been proposed as relevant in clinical-decision making for CAD prevention (332, 333) and were therefore chosen as the NRI cut-off points.

In the multivariate models in Studies I and II, a p-value of < 0.05 was considered significant. Otherwise, a more stringent level of significance ($p<0.01$) was chosen in order to correct for multiple testing. Statistical analyses were performed using SPSS 12.0.1, 15.0, PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA), Statistical Analysis System version 9.2 (SAS Institute, Cary, NC, USA), STATA Data Analysis and Statistical Software (StataCorp LP, College Station, TX, USA), and MedCalc (MedCalc Software bvba, Ostend, Belgium).

6 RESULTS

6.1 Associations between lipid profiles, AER, and eGFR (Study I)

Patients with impaired renal function had higher total cholesterol, LDL cholesterol, triglycerides, and ApoB and lower HDL cholesterol than patients with normal or only mildly impaired renal function (Table 4). The lipid profiles of patients with mildly impaired renal function were similar to those with normal renal function.

When patients were divided by their albuminuria status, lipid abnormalities could be seen already at the microalbuminuric stage for total cholesterol, triglycerides, and ApoB ($p<0.001$ for all). In macroalbuminuric patients, the lipid disturbances were further altered with higher total cholesterol, LDL cholesterol, triglycerides, and ApoB as well as with lower HDL, HDL_2, and HDL_3 cholesterol than in both normo- and microalbuminuric patients ($p<0.001$ for all).

Table 4. Lipid profile stratified by estimated glomerular filtration rate (eGFR).

	eGFR >90	eGFR 60-90	eGFR <60
N	1505	857	228
Total cholesterol (mmol/l)	4.80 ± 0.95	5.04 ± 0.84	5.37 ± 1.10‡*
Non-HDL cholesterol (mmol/l)	3.51 ± 0.96	3.64 ± 0.89	4.12 ± 1.04‡*
LDL cholesterol (mmol/l)	2.98 ± 0.86	3.14 ± 0.80	3.40 ± 0.92‡*
Triglycerides (mmol/l)	1.00 (0.76-1.41)	0.95 (0.74-1.28)	1.38 (1.04-2.10)‡*
Apolipoprotein B (g/l)	0.87 ± 0.22	0.88 ± 0.21†	0.99 ± 0.25‡*
HDL cholesterol (mmol/l)	1.27 ± 0.34	1.40 ± 0.38	1.19 ± 0.39‡*
HDL_2 cholesterol (mmol/l)	0.50 ± 0.23	0.61 ± 0.27	0.49 ± 0.26‡*
HDL_3 cholesterol (mmol/l)	0.77 ± 0.18	0.79 ± 0.21	0.71 ± 0.20‡*
Apolipoprotein A-I (g/l)	1.36 ± 0.21	1.44 ± 0.22†	1.37 ± 0.23*

Data are means ± SD or median (IQR). †$p<0.01$, ‡$p<0.001$ vs. eGFR >90; *$p<0.001$ vs. eGFR 60–90. Data are adjusted for age, body mass index, and sex.

Factors associated with eGFR and AER in multiple linear regression models

To study factors associated with eGFR, multiple linear regression analysis was performed. Age, BMI, ApoB, and SBP were independently associated with eGFR (R^2=0.28) (Table 5). When ApoB was replaced with triglycerides and LDL cholesterol, triglycerides were also independently associated with eGFR (R^2=0.28). When AER was added to the model with ApoB, systolic blood pressure and ApoB were no longer independently associated, but HDL cholesterol emerged as a new independently associated factor together with age, BMI, and AER (R^2=0.36).

Table 5. Factors associated with eGFR in a multiple linear regression analysis.

	B	SE	β	p-value
Age (years)	−1.17	0.05	−0.45	<0.001
Systolic blood pressure (mmHg)	−0.13	0.03	−0.08	<0.001
Body mass index (kg/m^2)	2.18	0.15	0.26	<0.001
Apolipoprotein B (g/l)	−10.39	2.34	−0.08	<0.001

R^2=0.28. Patients with lipid-lowering treatment were excluded. The model also included HDL cholesterol. eGFR= estimated glomerular filtration rate, B = unstandardized regression coefficient, SE = standard error of B, β = standardized regression coefficient.

SBP, HbA_{1c}, diabetes duration, ApoB, and HDL cholesterol were independently associated with AER (R^2=0.23) (Table 6). When ApoB was replaced with triglycerides and LDL cholesterol, they were also independently associated with AER (R^2=0.23). When eGFR was added to the model with ApoB, it was also an independent factor for AER together with all of the other variables in the model (R^2=0.27).

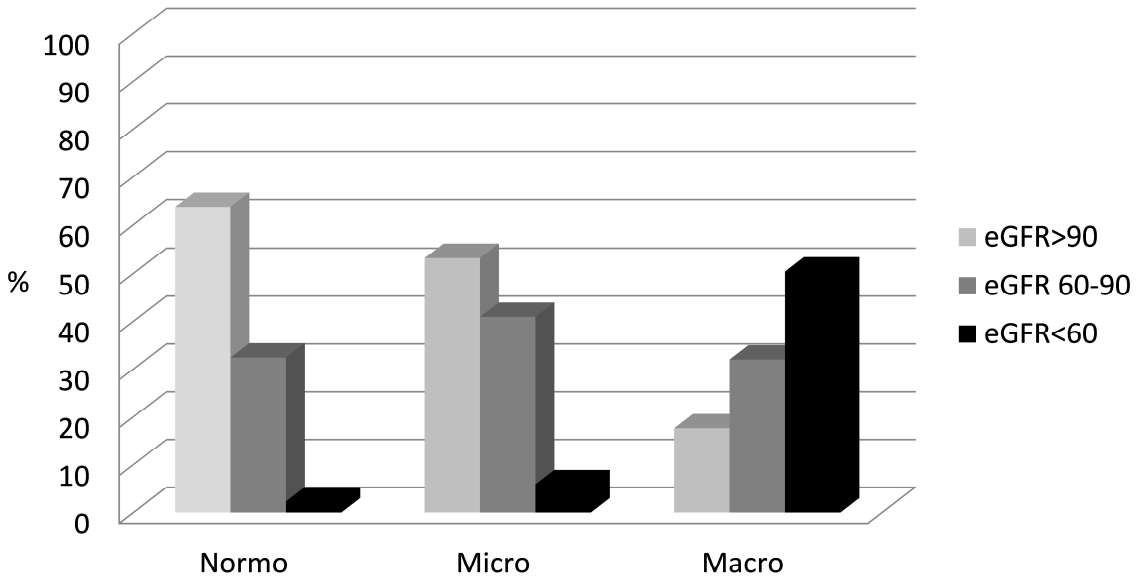
Table 6. Factors associated with ln AER in multiple linear regression analysis.

	B	SE	β	p-value
Diabetes duration (years)	0.02	0.003	0.16	<0.001
Systolic blood pressure (mmHg)	0.02	0.002	0.21	<0.001
HbA_{1c} (%)	0.23	0.02	0.20	<0.001
HDL cholesterol (mmol/l)	-0.39	0.09	-0.09	<0.001
Apolipoprotein B (g/l)	1.29	0.16	0.17	<0.001

R^2=0.23. Patients with lipid-lowering treatment were excluded. Ln AER= natural logarithm of albumin excretion rate, B = unstandardized regression coefficient, SE = standard error of B, β = standardized regression coefficient.

The relationship between eGFR and AER can be seen in Figures 10A and 10B.

A



B

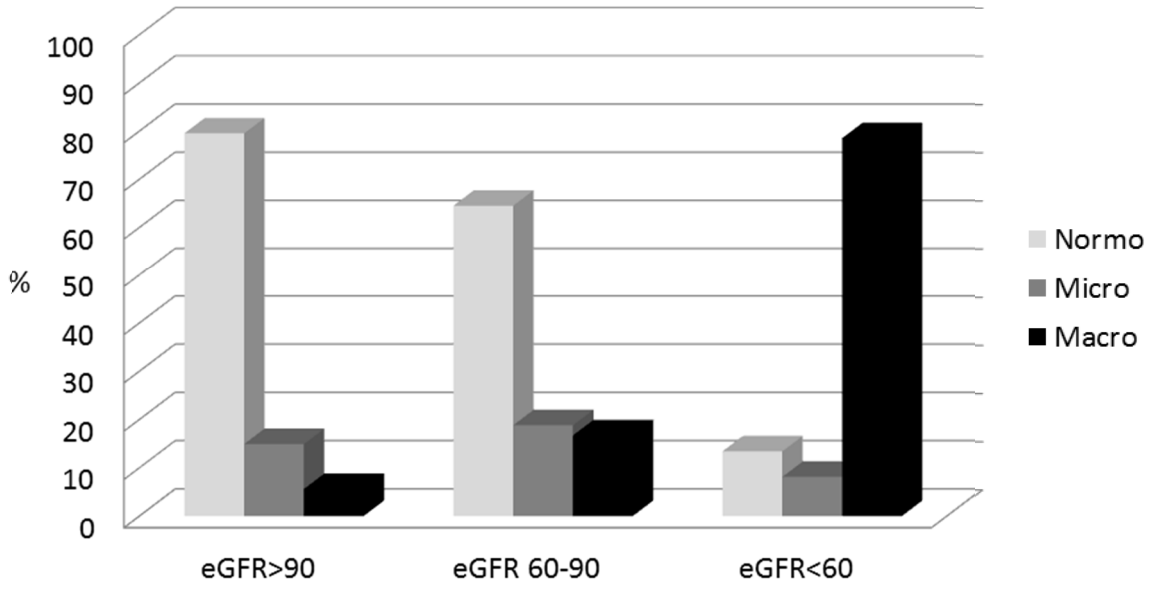


Figure 10. A) Frequency of normal (eGFR >90), mildly impaired (eGFR 60-90), or impaired (eGFR <60) renal function when patients are stratified by albuminuria status. B) Frequency of normal albumin excretion rate (normo), microalbuminuria (micro), or macroalbuminuria (macro) when patients are stratified by renal function. eGFR= estimated glomerular filtration rate.

Prevalence of patients achieving the targets for lipid variables

During the time of the data collection, 1994-2004, two targets for LDL cholesterol were in use. In patients without manifest renal disease who had good glycemic control, normal blood pressure, or normal body weight, only 51% achieved the criteria for an LDL cholesterol target of ≤3.0 mmol/l and merely 41% the more stringent criteria of ≤2.6 mmol/l. In patients with renal disease, the treatment targets were achieved by even fewer patients (Figure 11).

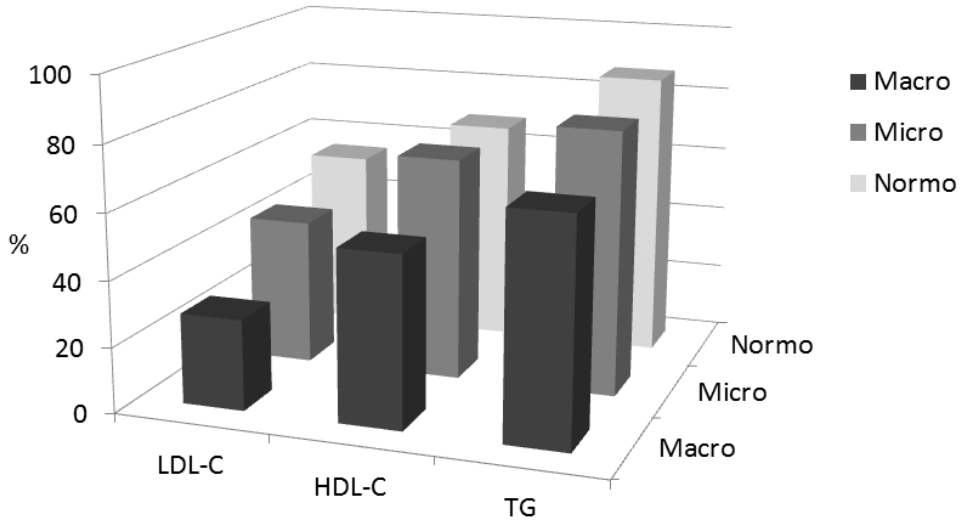


Figure 11. Prevalence of patients stratified by their albuminuria status reaching recommended targets of LDL cholesterol (≤3.0 mmol/l) HDL cholesterol (≥1.0 mmol/l for men, ≥1.3 mmol/l for women), and triglycerides (≤1.7 mmol/l). Normo = normal albumin excretion rate, micro = microalbuminuria, macro = macroalbuminuria, -C= cholesterol, TG= triglycerides.

6.2 Prediction of progression of renal disease by lipid profiles (Study II)

During a follow-up of 5.4 ± 2.0 years, 100 patients developed microalbuminuria, 50 progressed from micro- to macroalbuminuria, and 92 progressed from macroalbuminuria to ESRD. Hence, 242 (10.5%) of 2304 patients progressed to a higher level of albuminuria or developed ESRD.

Patients who developed microalbuminuria or progressed from micro- to macroalbuminuria had higher total cholesterol, non-HDL cholesterol, triglycerides, ApoB, and triglyceride/HDL cholesterol ratio ($p<0.001$ for all) at baseline than patients who did not progress.

Progressors from macroalbuminuria to ESRD had higher total cholesterol, LDL cholesterol, non-HDL cholesterol, triglycerides, ApoB, ApoB/ApoA-I, and triglyceride/HDL cholesterol ratio ($p<0.001$ for all), as well as lower HDL, HDL_2,

HDL_2/HDL_3 cholesterol ratio and ApoA-II concentrations ($p<0.01$ for all) than patients who did not progress.

Lipid variables as independent predictors for progression of renal disease

In a Cox regression analysis, HbA_{1c}, male sex, and triglycerides were independent predictors of development of microalbuminuria and progression to macroalbuminuria. When triglycerides were replaced with the other lipid variables one at a time, ApoB was also an independent predictor of progression to both micro- and macroalbuminuria. When AER was added to the original models, triglycerides were no longer an independent predictor of progression to micro- or macroalbuminuria. However, when the two groups were pooled; HbA_{1c}, male sex, triglycerides, and AER were all independent predictors of progression of renal disease.

High SBP, low BMI, and high triglycerides were predictive of progression from macroalbuminuria to ESRD. Total cholesterol, non-HDL cholesterol and triglyceride/HDL cholesterol ratio were also strong predictors of progression to ESRD ($p<0.001$). However, when baseline eGFR was included in the models, only total cholesterol predicted progression to ESRD together with HbA_{1c} and eGFR.

When ROC curves were created no thresholds for triglycerides and progression of renal disease were identified. To determine at which triglyceride concentration the risk for renal disease progression increases, we also prepared predictive probability plots for progression from normo- to microalbuminuria and from macroalbuminuria to ESRD (Figure 12). The risk of progression increased linearly until a triglyceride concentration of 4 mmol/l was reached and no clinically relevant thresholds for triglycerides were seen.

A

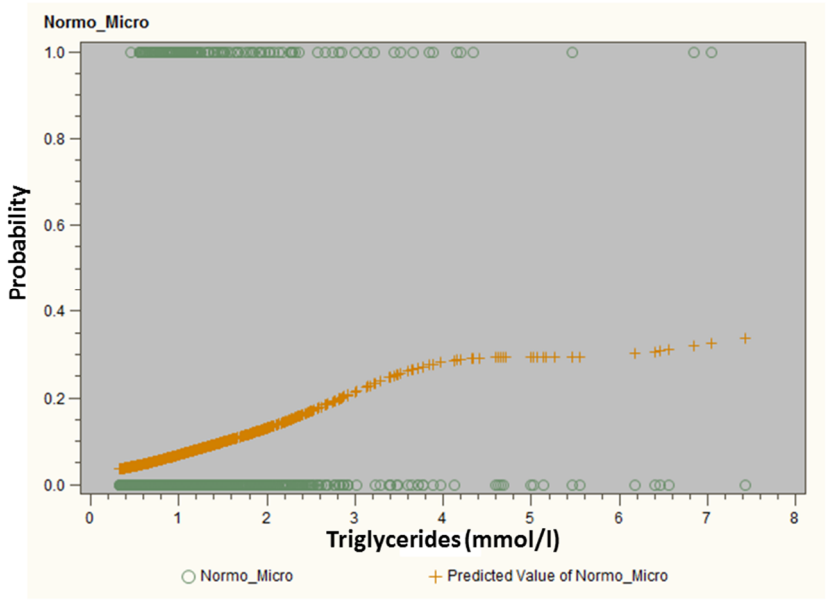


Figure 12A. Predicted probability plots for triglyceride concentrations and progression from normal AER to microalbuminuria.

B

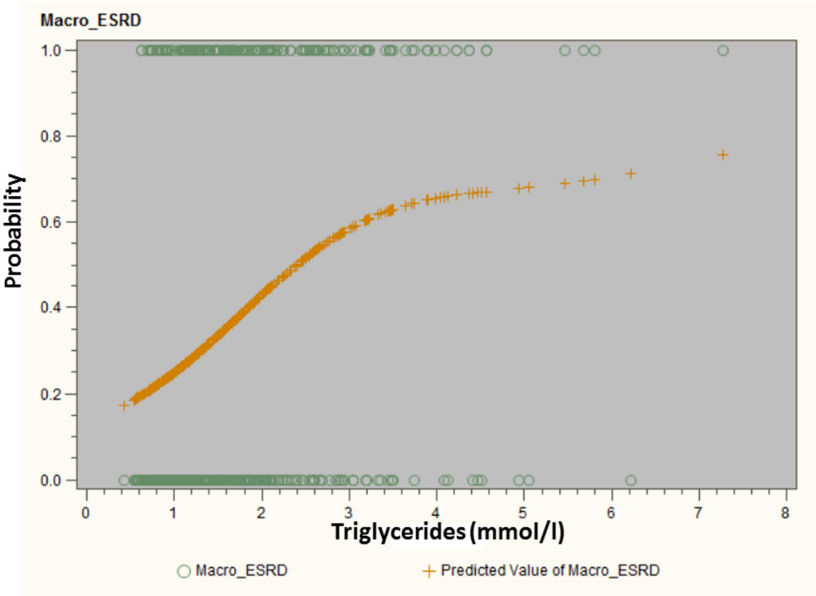


Figure 12B. Predicted probability plots for triglyceride concentrations and progression from macroalbuminuria to end-stage renal disease.

6.3 Associations between lipid variables, diabetic retinopathy, and nephropathy (Study III)

Of 1465 patients, 381 had no signs of retinopathy, 405 had mild NPDR, 186 had moderate to severe NPDR, and 493 had PDR. Total cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, and ApoB were higher in patients with PDR and NPDR than in patients without retinopathy ($p<0.001$ for all). HDL, HDL_2, and HDL_3 cholesterol were also lower in patients with PDR than in patients without retinopathy or with NPDR in both men and women ($p<0.001$ for all).

Diabetes duration and ln AER were positively associated, whereas eGFR and HDL cholesterol (odds ratio [OR] 0.45 [95% CI 0.27-0.74] p=0.002, R^2=0.48) were negatively associated with PDR in logistic regression analysis. When HDL cholesterol was replaced with other lipid variables one at a time, HDL_2 cholesterol (OR 0.29 [95% CI 0.14-0.60] p=0.001, R^2=0.48) was also inversely associated with PDR. Diabetes duration, HbA_{1c}, ln AER, and ln triglycerides (OR 1.86 [95% CI 1.18-2.93] p=0.008, R^2=0.44) were positively associated and eGFR was negatively associated with mild NPDR. Patients with PDR or moderate to severe NPDR were excluded from these analyses. When triglycerides were replaced with the other lipid variables, ln triglyceride/HDL cholesterol ratio (OR 1.62 [95% CI 1.14-2.31] p=0.008, R^2=0.44) was also positively associated with mild NPDR.

When patients without any signs of renal disease (i.e. normal AER or eGFR >60ml/min/1.73 m^2) were analyzed separately, total cholesterol/HDL cholesterol ratio differed significantly between the retinopathy status groups (p=0.007). Trends towards differences in total cholesterol, non-HDL, HDL and HDL_3 cholesterol and triglyceride/HDL cholesterol ratio could also be seen (p<0.05).

Significant interactions between retinopathy status and triglycerides, non-HDL cholesterol, ApoB, total cholesterol/HDL cholesterol ratio, and triglyceride/HDL cholesterol ratio (p<0.001 for all) as well as total cholesterol (p=0.006) were found when interaction terms between retinopathy status (no retinopathy, mild NPDR, moderate to severe NPDR, PDR) and lipid variables were calculated in linear regression models with ln AER as the dependent variable. Further, no significant correlations between the lipid variables and AER were seen in patients without diabetic retinopathy, whereas the correlations between AER and most of the lipid variables were strong in patients with moderate to severe NPDR or PDR (Table 7).

Table 7. Spearman correlations between AER and lipid variables according to retinopathy status.

	No retinopathy	Mild NPDR	Moderate to severe NPDR	PDR
N (men/women)	141 (64/77)	320 (137/183)	144 (97/47)	318 (160/158)
Total cholesterol	NS	NS	0.20 (p=0.02)	0.21 (p<0.001)
Non-HDL cholesterol	NS	NS	0.27 (p=0.001)	0.24 (p<0.001)
LDL cholesterol	NS	NS	0.17 (p=0.04)	0.14 (p=0.02)
Triglycerides	NS	0.18 (p=0.001)	0.35 (p<0.001)	0.36 (p<0.001)
Apolipoprotein B	NS	0.16 (p=0.004)	0.36 (p<0.001)	0.25 (p<0.001)
HDL cholesterol	NS	NS	-0.21 (p=0.01)	-0.17 (p=0.002)
Apolipoprotein A-I	NS	NS	NS	NS
Total chol/HDL chol	NS	NS	0.29 (p<0.001)	0.26 (p<0.001)
Triglyceride/HDL chol	NS	0.14 (p=0.01)	0.33 (p<0.001)	0.34 (p<0.001)
Apolipoprotein B/A-I	NS	NS	0.29 (p<0.001)	0.18 (p=0.001)

Patients with duration of diabetes less than 10 years and patients with end-stage renal disease were excluded. AER= albumin excretion rate, NPDR = non-proliferative diabetic retinopathy, PDR = proliferative diabetic retinopathy, Chol = cholesterol, NS = non-significant.

6.4 Ability of lipid variables to predict incident CAD events (Study IV)

Of 3520 patients, 310 (9%) suffered an incident CAD event during a median of 10.2 (8.6-12.0) years of follow-up. In general, patients who had an incident CAD event were older, had a longer duration of diabetes, higher SBP, WHR, and AER as well as lower eGDR and eGFR. Of the lipid variables, total cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, and ApoB were higher and HDL cholesterol was lower in patients with a CAD event than in those without (Table 8).

Table 8. Lipid profile stratified by an incident coronary artery disease (CAD) event.

	No CAD event	CAD event
N	3,037	310
Total cholesterol (mmol/l)	4.86 ± 0.92	5.42 ± 1.20‡
Non-HDL cholesterol (mmol/l)	3.51 ± 0.96	4.20 ± 1.23‡
LDL cholesterol (mmol/l)	2.97 ± 0.83	3.45 ± 1.00‡
Triglycerides (mmol/l)	0.98 (0.74-1.39)	1.30 (0.97-1.89)‡
Apolipoprotein B (g/l)	0.86 ± 0.22	1.00 ± 0.25‡
HDL cholesterol (mmol/l): Men	1.24 ± 0.34	1.14 ± 0.34‡
Women	1.46 ± 0.39	1.33 ± 0.41‡
HDL_2 cholesterol (mmol/l): Men	0.47 ± 0.24	0.44 ± 0.23‡
Women	0.64 ± 0.28	0.57 ± 0.28‡
HDL_3 cholesterol (mmol/l): Men	0.78 ± 0.19	0.70 ± 0.18‡
Women	0.83 ± 0.22	0.76 ± 0.22†
Apolipoprotein A-I (g/l): Men	1.32 ± 0.20	1.32 ± 0.20*
Women	1.46 ± 0.23	1.41 ± 0.23†
Apolipoprotein B/A-I	0.63 ± 0.20	0.75 ± 0.23‡
Total cholesterol/HDL cholesterol	3.58 (2.91-4.46)	4.30 (3.55-5.60)‡
Triglyceride/HDL cholesterol	0.76 (0.51-1.17)	1.06 (0.73-1.90)‡

Data are means ± SD or median (IQR). †p<0.01, ‡p<0.001. P-values are adjusted for age, body mass index, and sex (if not already stratified by sex). * Apolipoprotein A-I concentrations in men were 1.27 and 1.33 g/l in no CAD and CAD event groups, respectively, when corrected for age and body mass index, and this difference was significant.

To take into account the competing event of non-CAD death, we performed Fine and Gray regression analysis (Table 9) in addition to the Cox regression analysis. Duration of diabetes, eGFR, ApoB, SBP, and laser treatment were all independent predictors of CAD in both analyses. In the entire cohort, ApoB, triglycerides, non-HDL cholesterol, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio were the strongest lipid predictors of an incident CAD event.

Table 9. Competing risk regression analysis with risk factors for an incident CAD event.

	SubHR (95% CI)	p-value
eGFR (28 ml/min/1.73 m^2)	0.69 (0.58 - 0.84)	<0.001
Diabetes duration (11.8 years)	2.39 (2.01 - 2.84)	<0.001
Apolipoprotein B (0.23 g/l)	1.40 (1.19 - 1.64)	<0.001
Laser treatment (yes, no)	1.61 (1.15 - 2.25)	0.005
Systolic blood pressure (18 mmHg)	1.22 (1.06 - 1.40)	0.005

Results are presented as subdistribution hazard ratios (sub)HRs per SD increase with 95% CI. The model also included HbA_{1c}, sex, waist-to-hip ratio, natural logarithm of albumin excretion rate, and history of smoking. Coronary artery disease (CAD) events = 198, controls = 2219 and non-CAD deaths = 104.

The percentage of patients with a history of smoking was high, and this could be a confounding factor. History of smoking at baseline was univariately associated with CAD, whereas current smoking was not, and therefore, we chose to include history of smoking in the multivariate models. In the multivariate model, history of smoking was not independently associated with CAD events in the entire cohort, unlike several of the lipid variables. We also performed additional NRI analyses to look at the predictive value of history of smoking versus ApoB. The NRI was 0.6% (p=0.62) when we added history of smoking to the multivariate model (including duration of diabetes, eGFR, SBP, retinal laser treatment, sex, HbA_{1c}, WHR, AER, and ApoB), whereas it was 7.7% (p=0.01) when ApoB was added to the model.

Different lipid variables predicted an incident CAD event when patients were divided by sex, renal status, and HbA_{1c}. In men, ApoB was the only lipid variable that was an independent predictor of CAD, whereas in women triglycerides, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio were the strongest predictors. In macroalbuminuric patients, ApoB and non-HDL, LDL, and total cholesterol were the strongest predictors, whereas ApoB/ApoA-I ratio, triglyceride/HDL cholesterol ratio, triglycerides, and ApoA-I preformed the best in patients with normal AER. ApoB/ApoA-I, triglyceride/HDL cholesterol ratio, and triglycerides were also the best predictors in patients with an HbA_{1c} below the median of the cohort (8.3%), whereas in patients with an HbA_{1c} above the median, the same lipid variables as in the macroalbuminuric patients (ApoB, non-HDL cholesterol, LDL cholesterol, and total cholesterol) were the strongest predictors.

To examine clustering of risk factors, we divided the patients into five groups according to the number of risk factors present. In Cox regression analysis with CAD events as the dependent and clustering as the independent variable, the HR was not significantly different from that of the reference group (i.e. those patients without any or with only one of the five risk factors) for patients with no more than two of any of the five risk factors.

However, in patients with three or more risk factors, the rise in hazard ratio was clear (Figure 13).

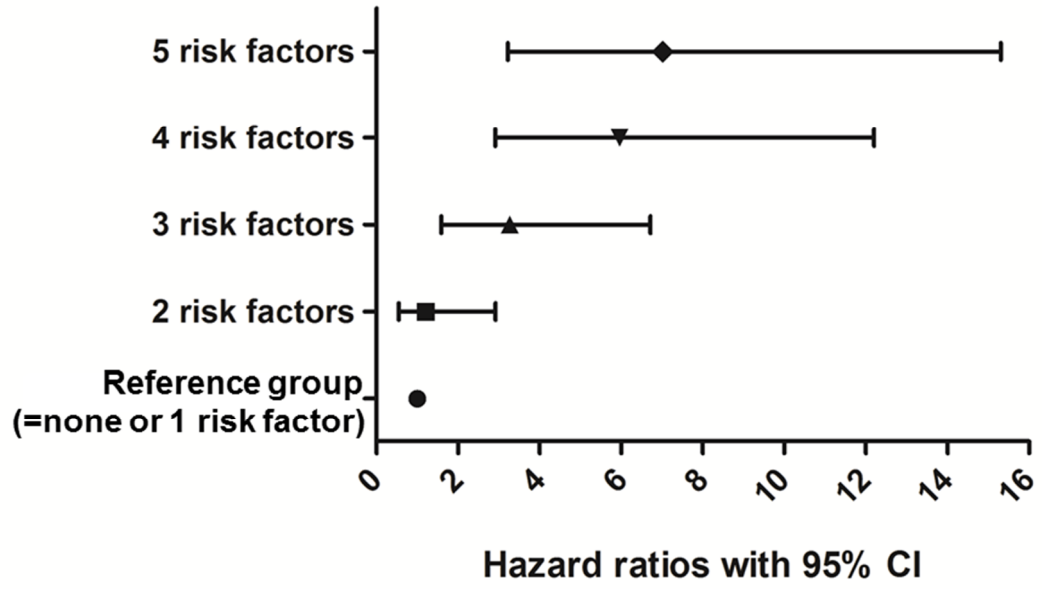


Figure 13. Cox regression analysis for clusters of risk factors for CAD events. Patients were stratified into groups according to the presence of any of the five risk factors listed below at baseline: 1) Hypertension, defined as either a systolic blood pressure >140 mmHg or a diastolic blood pressure >80 mmHg (117), 2) presence of renal disease, defined as presence of microalbuminuria, macroalbuminuria, or end-stage renal disease, or an estimated glomerular filtration rate <60 ml/min/1.73 m^2, 3) exceeding the recommended HbA_{1c} >7.0% (117), 4) current smoking, or 5) dyslipidemia, defined as total cholesterol >5.0 mmol/l, LDL cholesterol >2.6 mmol/l, triglycerides >1.7 mmol/l, HDL cholesterol <1.0 mmol/l for men, HDL cholesterol <1.3 mmol/l for women (117, 334), or apolipoprotein B > 0.90 g/l (335).

7 DISCUSSION

7.1 Association between renal disease and lipid profiles

7.1.1 Lipid profiles and eGFR

Patients with type 1 diabetes without complications and good glycemic control often have similar or even more favorable lipid profiles than the background population (336, 337). However, we found that patients with type 1 diabetes with an impaired renal function (eGFR<60 ml/min/1.73 m^2) had higher triglycerides, total cholesterol, LDL cholesterol, and ApoB, and lower HDL cholesterol than patients with eGFR above 60 ml/min/1.73 m^2. Triglycerides were an independent predictor of eGFR in a multiple linear regression model, whereas LDL cholesterol and HDL cholesterol were not. When triglycerides and LDL cholesterol were replaced with ApoB, it was also an independent predictor of eGFR. Previous studies are scarce, but after the publication of our study, associations between lower HDL cholesterol and impaired renal function were observed in patients with type 1 or type 2 diabetes (338). Higher triglycerides were also seen in patients with type 2 diabetes. In patients with type 2 diabetes, but without macroalbuminuria, higher ApoB/LDL cholesterol ratio and ApoC-III concentrations have been associated with impaired renal function regardless of the presence of microalbuminuria (339). In a Korean study of the general population with 93 228 participants, triglycerides and triglyceride/HDL cholesterol ratio were the strongest lipid variables associated with impaired renal function in men, whereas in women LDL cholesterol and non-HDL cholesterol showed the highest ORs (340).

7.1.2 Lipid profiles and AER

The association between lipid variables and AER in patients with type 1 diabetes has been examined to a larger extent. In line with our study, triglycerides and total cholesterol were found to be associated with macroalbuminuria in the DCCT/EDIC, EURODIAB and Estudio Diamante cohorts (144, 341, 342). LDL cholesterol was not reported in the Estudio Diamante Study, but in the other two studies it was also significantly associated with macroalbuminuria. In our study, HDL cholesterol was significantly lower in patients with macroalbuminuria, whereas no significant differences were seen between patients with normal AER or microalbuminuria. In the EURODIAB Study, an association between macroalbuminuria and HDL cholesterol was only seen in women and in the DCCT/EDIC Study, HDL cholesterol was not associated with AER in a multivariate model. We found that triglycerides, total cholesterol, and ApoB were all significantly higher in patients with microalbuminuria. In contrast, in the EURODIAB Study, triglycerides were the only lipid variable to remain significantly associated with

microalbuminuria after correcting for multiple confounding factors. ApoB was not measured in the EURODIAB or Estudio Diamante Studies, but in the DCCT/EDIC Study it was significantly associated with AER in a multivariate model. Hence, while dyslipidemia is more evident in advanced diabetic nephropathy, it can be seen to a lesser degree already at the stage of microalbuminuria.

7.1.3 Normoalbuminuric renal impairment

We observed that 13.4% of patients with eGFR below 60 ml/min/1.73 m^2 had normal AER and 2.3% of patients with normal AER had an eGFR below 60 ml/min/1.73 m^2 (Figure 10A and 10B). In previous studies, the frequency of normoalbuminuric renal impairment in patients with type 1 diabetes has generally varied between 8.3% and 24% (88, 343, 344). In type 2 diabetes, the most recent studies have found that 36-57% of patients with impaired renal function are normoalbuminuric (338, 345-349). The frequency of this condition has increased over the years (350), which may be explained by the use of more effective medications to treat hypertension (i.e. ACE inhibitors and ARBs) and hyperglycemia.

It has been speculated that in patients with type 2 diabetes, causes other than the classical diabetic glomerulosclerosis contribute to the development of normoalbuminuric renal impairment, e.g. ischemic vascular disease, interstitial fibrosis, or cholesterol microembolism (351). In support of this, retinopathy and albuminuria were both absent in as many as 30% of patients with type 2 diabetes and impaired renal function, indicating other causes of renal disease than true diabetic nephropathy (351). Moreover, resistance of the intrarenal arteries has been shown to be increased in patients with type 2 diabetes and impaired renal function, irrespective of albuminuria status (352). However, patients with type 1 diabetes present a more homogeneous renal phenotype, and the frequency of other causes of renal disease is substantially lower (353). Further, more advanced diabetic glomerular lesions were observed in normoalbuminuric patients with type 1 diabetes and reduced GFR (<90 ml/min/1.73 m^2) than in those with normal GFR (343). Therefore, normoalbuminuric renal impairment cannot be solely explained by other causes of renal disease than diabetic nephropathy. Patients with normoalbuminuric renal impairment are more frequently women, have less retinopathy, are older, and have shorter diabetes duration than patients with albuminuric renal impairment (338, 345, 346).

The clinical significance of normoalbuminuric renal impairment has been debated, and the trait has been suggested to be explained by physiological aging. However, even if normoalbuminuric renal impairment is likely to be more benign than albuminuric renal impairment, it is still associated with a significant CVD burden (346), and both eGFR and AER have been found to be independently associated with mortality and progression to ESRD (354).

7.1.4 Achievement of recommended lipid targets

Few patients reached the internationally recommended lipid targets in our study. The targets were especially poorly met in patients with albuminuria, in patients with impaired renal function, and in patients without manifest renal disease who had poor glycemic control, were overweight, or were hypertensive. Our data suggest that many patients with type 1 diabetes are in need of lipid-lowering treatment for the prevention of CVD.

7.2 Lipid variables as predictors of progression of renal disease

Lipid abnormalities predicted progression of diabetic nephropathy at all stages of renal disease. When progression from normo- to microalbuminuria and micro- to macroalbuminuria was pooled, the overall progression of renal disease was predicted by triglycerides independently of other risk factors, including both AER and eGFR. When progression to micro- and macroalbuminuria was analyzed separately, triglycerides predicted development of incident microalbuminuria or progression to macroalbuminuria, but when AER was entered into the models triglycerides were no longer an independent predictor. The definition of renal disease may influence the results. Progression to micro- or macroalbuminuria was defined based on a change in the degree of albuminuria, and therefore, AER is already by definition a very strong predictor of progression. The power was naturally also better in the pooled analysis, especially regarding progression from micro- to macroalbuminuria, since only 50 patients progressed from micro- to macroalbuminuria during the follow-up period. ApoB also predicted progression of both micro- and macroalbuminuria in their respective analyses, but like triglycerides, not independently of AER.

In the EURODIAB Study, the triglyceride concentration was almost as strong a predictor as AER for the development of microalbuminuria, with a standardized estimate of relative risk (SERR) of 1.3 compared with 1.5 for AER (355). Regarding progression to macroalbuminuria, triglycerides were not an independent predictor of progression, but in the univariate analyses triglycerides were significantly higher in patients who progressed to macroalbuminuria and the lowest concentrations were seen in the group that regressed to normoalbuminuria (356). In the DCCT/EDIC Study, triglycerides, total cholesterol, and LDL cholesterol were all associated with progression to macroalbuminuria and regression to normoalbuminuria (357). In the Pittsburgh EDC Study, LDL cholesterol predicted development of microalbuminuria in men and in all patients combined, whereas triglycerides predicted development of microalbuminuria in women and in patients with diabetes duration of over 20 years (134). In another follow-up study of the same cohort, triglycerides, LDL cholesterol, and non-HDL cholesterol predicted progression to overt nephropathy (defined as macroalbuminuria or ESRD) within 5 years, but not in patients who progressed 6-10 years after baseline (125). In the German Diabetes Documentation System Study, triglycerides and LDL cholesterol were significant risk factors for the development of microalbuminuria. Furthermore, dyslipidemia, defined as at least one lipid

variable above the cut-off thresholds (total cholesterol >200 mg/dl ≈ 5.2 mmol/l, LDL cholesterol >160 mg/dl ≈ 4.1 mmol/l, or triglycerides >150 mg/dl ≈ 1.7 mmol/l), was associated with progression to macroalbuminuria (358).

In our study, several lipid variables predicted progression from macroalbuminuria to ESRD, e.g. high total cholesterol, triglycerides, LDL cholesterol, non-HDL cholesterol, and ApoB/ApoA-I ratio as well as low HDL cholesterol. However, when baseline eGFR was entered into the model, the only lipid variable that remained an independent predictor was total cholesterol. In a cohort from the Steno Diabetes Center including patients with type 1 diabetes and macroalbuminuria at baseline, total cholesterol was significantly associated with a decline in GFR measured with the Cr-EDTA plasma clearance technique (359). Triglycerides predicted renal failure in the World Health Organization Multinational Study of Vascular Disease in Diabetes (WHO-MSVDD) in patients with type 2 diabetes, but not in patients with type 1 diabetes (360). However, the power was much lower in patients with type 1 diabetes (only 53 vs. 134 events in patients with type 1 and type 2 diabetes, respectively), and triglycerides were only measured in a subset of the participating study centers, decreasing the power even further. In the DCCT/EDIC Study, lipid variables did not predict the development of impaired eGFR (<60 ml/min/1.73 m^2) (357), however, in the Swedish National Diabetes Register (NDR) Study of patients with type 2 diabetes, triglycerides were independently associated with the development of impaired eGFR (361).

When we divided the patients into quartiles of the triglycerides, the highest quartile had the highest HR for progression of renal disease at all stages (see Figure 1 in Study II). Moreover, the hazard ratio for the development of incident microalbuminuria increased significantly with every quartile. It is noteworthy that the cut-off levels for the triglyceride quartiles were much lower than the recommended cut-off threshold of <1.7 mmol/l in the current treatment guidelines. In ROC curve analyses of triglycerides for the prediction of renal disease development and progression (see Study II Supplementary Figures 1A-C) and in Figures 12A and 12B, no clinically relevant thresholds for triglycerides with regard to the progression of renal disease emerged.

It is, however, difficult to ascertain causal links based on these data since repeated lipid and AER measurements from the day of diabetes diagnosis are not available. Whether the lipid abnormalities are primary and consistently precede the development of renal disease or are merely a consequence of renal disease has been widely debated and remains unknown. However, based on the findings from the above prospective studies, and the fact that favorable lipid profiles are associated with regression of microalbuminuria (362) and dyslipidemia with faster progression of renal disease (125, 363-366), there is an evident clinical message: dyslipidemia is associated with a poorer prognosis, especially if other risk factors, such as hyperglycemia, hypertension, smoking, and obesity, are simultaneously present.

7.3 Lipid profiles and diabetic retinopathy

In univariate analyses, multiple associations between lipid variables and PDR emerged, but in the multivariate models after correction for confounding factors, e.g. renal disease and HbA_{1c}, only HDL cholesterol and HDL_2 cholesterol were independently associated with PDR. When only patients without retinopathy or mild NPDR were included in the analyses, triglycerides and triglyceride/HDL cholesterol ratio were associated with mild NPDR independently of confounding factors. These results are in line with some of the previous studies. In the EURODIAB Study, triglycerides were independently associated with both moderate to severe NPDR and PDR (20). In the DCCT/EDIC Study, the severity of retinopathy was inversely associated with HDL cholesterol and VLDL size and positively associated with triglycerides and small- and medium-sized VLDL (367). Some earlier studies report significant associations between diabetic retinopathy and total cholesterol or LDL cholesterol (17, 368), but we only found significant associations in the univariate analyses. However, there are also several studies in which no associations between lipid variables and retinopathy status have been found (18, 19, 369).

Among prospective studies, triglycerides were independently associated with the development of retinopathy in the EURODIAB Study (370) and with the progression of retinopathy in the Pittsburgh EDC Study (371). However, lipid variables were not predictive of progression of PDR in the Sorbinil Retinopathy Trial (372) nor in the DCCT Study (373).

It is difficult to compare these studies with each other because of differing methods for detection of diabetic retinopathy, differences in the definition of retinopathy, differences in sizes of the study cohorts, and differing degree of nephropathy and availability of other risk factors for multivariate models. Relative to the strength of the associations between hyperglycemia and retinopathy and between lipid variables and CVD, the associations between lipids and retinopathy are clearly weaker, and it is impossible to draw any definite conclusions.

However, a consensus can be found regarding the association between serum lipid levels and hard exudates. Hard exudates are usually the consequence of lipid leakage from dysfunctional retinal capillaries and are considered an early sign of diabetic retinopathy and are also typically associated with maculopathy. In the WESDR Study, total cholesterol was significantly associated with the presence and severity of hard exudates (369), and in the ETDRS Study total and LDL cholesterol were also associated with hard exudates (374). In studies including mostly patients with type 2 diabetes, such as the Hoorn Study (375) and the Atherosclerosis Risk in Communities (ARIC) Study (376), associations between lipid variables and hard exudates were also confirmed.

The presence or absence of diabetic nephropathy is probably the most important confounding factor and a driving force behind the conflicting results in the studies exploring the associations between diabetic retinopathy and lipid variables. Similar

mechanisms and risk factors are thought to be behind the development of both diabetic retinopathy and nephropathy, and it is therefore no surprise that the two complications are strongly associated with each other (16). Diabetic nephropathy may lead to secondary changes in the lipid profile, and thus, it is difficult to determine whether the associations between retinopathy and the lipid variables are independent or they simply reflect the association between nephropathy and retinopathy. Therefore, multivariate models that take renal disease into account are needed, although one cannot be sure that this will eliminate the problem entirely. Notably, we found interactions between retinopathy, nephropathy, and several of the lipid variables. Due to our large cohort, we were also able to separately analyze patients with normal AER and eGFR >60 ml/min/1.73 m^2. Significant differences were seen in the total cholesterol/HDL cholesterol ratio by retinopathy status groups, and differences of borderline significance ($p<0.05$) emerged for several of the lipid variables. Clearly, associations between lipid variables and retinopathy are weaker when renal disease is taken into account, but there is also a risk for over-correction. Indeed 85% of our patients with PDR also had some signs of renal disease, and by excluding these patients from the analyses the group is no longer representative of the normal clinical setting.

To explore this dilemma from another angle, we looked at the correlations between AER and the lipid variables divided by the retinopathy status. In patients with moderate to severe NPDR or PDR, significant correlations between AER and the lipid variables were seen, as expected. However, in the patients without retinopathy, no significant correlations between AER and lipid variables were observed, and in the patients with mild NPDR only a few fairly weak correlations were present. Further, we found that in the patients without retinopathy or with only mild NPDR, AER was in fact low despite having unfavorable lipid profiles (i.e. HDL cholesterol in the lowest quartile or triglycerides, total cholesterol, or LDL cholesterol in the highest quartile). Importantly, retinopathy cannot cause secondary changes in the lipid profile so there has to be another explanation for this phenomenon. Possibly, some patients are protected from the unfavorable effects of lipid variables on microvascular complications. In contrast, the presence of severe retinopathy could serve as a marker for a more deleterious effect of hyperlipidemia and other risk factors on renal outcomes. In support of this theory, the EURODIAB Study found different associations between the blood pressure and AER in patients with and without retinopathy (377).

In light of these findings, it seems unwise to expect that the associations between lipid variables and retinopathy would be totally independent of renal disease. Furthermore, in contrast to HDL cholesterol, SBP was not independently associated with PDR when AER and eGFR were included in the multivariate model. Nevertheless, there is strong evidence for a causal relationship between blood pressure and progression of diabetic retinopathy from randomized clinical trials (165, 378).

Another problematic confounding factor is hyperglycemia. Associations exist between lipid variables and hyperglycemia, especially between triglycerides and hyperglycemia.

Again, multivariate models can take into account the confounding factors, but they cannot provide definite proof of an independent role of the factor studied. Moreover, it is not possible to draw any conclusions about a causal relationship from cross-sectional studies. Even prospective studies often have many confounding factors; hence, conclusions should be drawn with caution. Randomized clinical trials are needed to show a causal relationship. In the FIELD Trial, fenofibrate treatment reduced the need for the first laser treatment, and in the ACCORD Study patients who received fenofibrate on top of simvastatin treatment had a reduced rate of progression of diabetic retinopathy. Fenofibrate mainly reduces triglycerides and increases HDL cholesterol concentrations, however, in the FIELD Study the beneficial effect of fenofibrate did not seem to be related to any changes in the lipid concentrations. In the ACCORD Study, a clinically relevant decrease in triglycerides and increase in HDL cholesterol concentrations were seen in patients treated with fenofibrate compared with simvastatin alone, but the beneficial mechanism of fenofibrate remains unclear.

7.4 Lipid variables as predictors of a CAD event

In this study we showed that ApoB was the strongest independent predictor of an incident CAD event in the entire FinnDiane population. Triglycerides, non-HDL cholesterol, ApoB/ApoA-I ratio, and lipid ratios were also good predictors of an incident CAD event. Previous data from prospective studies in patients with type 1 diabetes are surprisingly scarce. In the EURODIAB Study, triglycerides and HDL cholesterol were independent predictors of CAD in separate models (379) after a 7-year follow-up. In the 10-year follow-up data from the Pittsburgh EDC Study, HDL cholesterol and non-HDL cholesterol predicted CAD events (380). In a Danish study, triglycerides, LDL cholesterol, and HDL cholesterol were all related to CAD after 13 years of follow-up (381). Finally, in the WHO-MSVDD Study, total cholesterol predicted the incidence of myocardial infarction in patients with type 2 diabetes, but not in patients with type 1 diabetes (382).

When men and women were analyzed separately, we found that ApoB was the strongest lipid predictor in men, whereas triglycerides, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio had the highest HR per SD increase in women (see Study IV Supplementary Table 4). In the EURODIAB Study, triglycerides also predicted CAD in women. However, in that study, apolipoproteins were not measured and lipid ratios were not calculated.

The predictive performance of LDL cholesterol in this study was poor. In the entire cohort, the NRI for LDL cholesterol was 3.2%, compared with 7.7% for ApoB. Moreover, in patients without renal disease, LDL cholesterol was not an independent predictor of CAD, and Figure 1A (in Study IV) shows that the median LDL cholesterol level was not able to separate cases from controls. Further, in patients with HbA_{1c} below 8.3% and in women, LDL cholesterol could not predict CAD independently of other risk factors.

Experimental studies have shown that remnant cholesterol accumulates in the arterial wall like LDL cholesterol (383, 384), and importantly, both ApoB and non-HDL cholesterol are capturing the risk of all the atherogenic particles, including the VLDL, IDL, and LDL. Of note, the mean baseline LDL cholesterol concentration (3.45 mmol/l) of patients with an incident CAD event in this study was lower than the mean LDL cholesterol concentrations in older clinical trials of lipid-lowering treatment (385). In addition, the presence of sdLDL particles is not revealed by LDL cholesterol concentrations. ApoB is a better detector of an increased number of sdLDL particles (see also Section 2.3.2), and in concordance, ApoB was a stronger predictor of CAD than LDL cholesterol in our study. Furthermore, already in children with type 1 diabetes a preponderance of sdLDL compared with children without diabetes has been reported (238). There are also several limitations regarding the LDL cholesterol calculated by the Friedewald formula. The best known is the underestimation of LDL cholesterol in hypertriglyceridemic conditions. LDL cholesterol should not be calculated using this formula in patients with triglyceride concentrations above 4.0 mmol/l, but as a consequence, several high-risk patients (N=48) could not be included in the multivariate models with LDL cholesterol as the lipid variable. Concerns have also been raised about the accuracy of the Friedewald LDL cholesterol at much lower triglyceride concentrations (386, 387) it has been found to underestimate LDL cholesterol concentrations already when triglycerides exceeds 1.7 mmol/l (388). Of note, also in previous prospective studies in patients with type 1 diabetes, other lipid variables have emerged as better predictors of CAD events than LDL cholesterol (379, 380). Data from randomized clinical trials have established LDL cholesterol lowering as the primary target of therapy for prevention of CAD, but the residual risk present beyond that of LDL cholesterol should be recognized in clinical practice, especially in patients with type 1 diabetes with fairly good glycemic control, in patients without renal disease, and in women.

In this study, HDL cholesterol was a weaker predictor of CAD than atherogenic lipid variables. Its best performance was seen in patients with normal AER at baseline; however, also in these patients, triglycerides and ApoA-I were stronger predictors of CAD. The relationship between HDL cholesterol and CAD is far more complex than initially assumed. Despite the strong inverse correlation between HDL cholesterol and CAD seen in epidemiological studies, a pharmacological increase of HDL cholesterol has failed to reduce the risk of CAD. Further, a Mendelian randomization study showed that a genetic mechanism that raised HDL cholesterol did not lower the risk of myocardial infarction, thereby calling into question the assumed causal relationship between low HDL cholesterol concentrations and CAD risk (389). From clinical practice, we know that low HDL cholesterol is rarely an isolated trait and is most often accompanied by increased triglycerides, abdominal obesity, and other components of metabolic syndrome. Therefore, we may have jumped to a conclusion by assuming a causal relationship between reduced HDL cholesterol and CAD risk. The poorer performance of HDL cholesterol compared with atherogenic variables could also be related to changes in functionality and a possible loss of protective effects of HDL cholesterol in patients with type 1 diabetes. The possible

functionality changes cannot be captured by the mere measurement of HDL cholesterol concentrations or lipid ratios, but interestingly, the lipid and lipoprotein ratios containing atherogenic and anti-atherogenic particles were among the strongest predictors of CAD in women and in healthier patients (i.e. patients without renal disease or with HbA_{1c} below 8.3%). By contrast, in patients with renal disease, in patients with HbA_{1c} above 8.3%, or in men, no additional benefit was gained from the ratios compared with the atherogenic lipid variables alone.

The frequency of patients with a history of smoking was high in the cohort, but the frequency of current smoking at baseline was much lower. Current smoking was not univariately associated with CAD events, whereas history of smoking was, and therefore history of smoking was chosen for the multivariate models. However, history of smoking was not an independent predictor of CAD, whereas several lipid variables were. Therefore, in this study smoking did not explain the high CAD risk any better than did lipid variables; however, both are definitely important risk factors that should be treated aggressively and are likely to have a potentiating effect. A dual increase in chronic inflammation could, for example, be one of the driving mechanisms for a potentiating effect.

When we performed a Cox regression analysis with clustering of risk factors as the independent variable, we could see that the rise in HR was not linear. For patients with no more than two of any of the five risk factors, the HR was not significantly different from that of the reference group. However, in patients with three risk factors the HR was already 3.27, in patients with four risk factors the HR was 5.96, and in those with all five risk factors the HR was 7.02 compared with the reference group. Our results indicate a potentiating effect with an increasing number of risk factors in patients with three or more risk factors.

The metabolic syndrome is a cluster of risk factors dominated by central obesity, increased triglycerides, decreased HDL cholesterol, and elevated blood pressure. In this study, eGDR, a formula for insulin sensitivity that includes WHR, HbA_{1c}, and hypertension, was clearly lower in patients with CAD than in controls (4.49 vs. 6.90 mg/kg/min). In the Pittsburgh EDC cohort, eGDR was shown to predict lower extremity arterial disease (390), macroalbuminuria (125), and hard CAD events (380).

7.5 Strengths and limitations

The patients in Studies I, II, and IV account for roughly 10% of all patients with type 1 diabetes in Finland and show even distribution geographically, closely following the distribution of the general population in Finland (see Figure 8). A selection bias is therefore less likely than in single hospital-based studies. Furthermore, the high response rate of 78% (325) makes any significant selection biases unlikely. An additional strength is that all lipid variables were measured in the same laboratory specializing in lipid

research and that the phenotypes for diabetic nephropathy, retinopathy, and CAD were robustly and meticulously assessed. Thus, this large cohort is unique for the detailed study of lipid profiles of patients with type 1 diabetes.

A limitation of Studies I and III was the cross-sectional study design; however, Study I was followed by a subsequent prospective study. In Study III, the retinopathy status was determined in 1465 consecutively recruited patients with complete lipid profiles. To avoid a selection bias, the patients were derived from the first patients participating in the FinnDiane Study. In Study II, the progression of renal disease was ascertained after a review of the medical files, and therefore, the follow-up information was not dependent on patients' participation in a revisit, only on their regular visits to their physician. In Study IV, the follow-up information on CAD events was obtained from the Finnish Hospital Discharge Register as well as the Causes of Death Register; hence, follow-up information was available for all patients. A limitation of the studies is the possible survival bias, however, this was accounted for by performing competing risk regression analyses in Study IV.

Another limitation of the study is that it is not feasible to measure the GFR directly in such a large cohort. In Study I, we used the Cockcroft-Gault formula (71). This formula was developed in a population of hospitalized men with a fairly wide range of renal function. Because the estimate also includes the tubular secretion of creatinine, it has been found to systematically overestimate the actual renal function (391). In Study II, we used both the Cockcroft-Gault and the MDRD-4 equation (72). The MDRD equation was developed in patients with chronic kidney disease. It has been validated in Caucasian populations aged between 18 and 70 years with eGFR <60 ml/min/1.73 m^2 and has shown good performance for patients with impaired renal function. However, the MDRD equation has been found to be less accurate in populations without impaired renal function (392, 393). The CKD-EPI equation (73) is the newest of the eGFR equations and was used in Study IV. It was developed in a population with a wide range of measured GFR to address the issue of underestimation of eGFR by the MDRD equation at eGFR levels above 60 ml/min/1.73 m^2. The CKD-EPI equation has been found to be as accurate as the MDRD equation when GFR is <60 ml/min/1.73 m^2 and more accurate when GFR is between 60 and 120 ml/min/1.73 m^2 (73).

Unfortunately, renal biopsies were not available from our patients. However, in patients with type 1 diabetes, the renal phenotype is much more homogeneous than in patients with type 2 diabetes (353) as previously discussed in Section 7.1.3. The presence of retinopathy is also considered to support the diagnosis of diabetic nephropathy (394). In our patients with ESRD, 98.5% had a history of laser treatment, which is performed to treat PDR or severe NDPR in the vast majority of patients (161).

7.6 Lipid variables and micro- and macrovascular complications

Triglycerides were associated with AER, eGFR, and mild NPDR and predicted the progression of renal disease at all stages of albuminuria and incident CAD events in the total population, in women, and in patients with normal AER. Triglyceride concentrations have been shown to be strongly related to glycemic control (337), and it is widely known that corrections of severe hyperglycemia will also lead to lower triglycerides. However, in all four studies, triglycerides were associated with or predicted adverse events independently of HbA_{1c}. Furthermore, triglycerides were not the only lipid variable associated with adverse microvascular events, for example, ApoB also predicted progression to micro- and macroalbuminuria, total cholesterol predicted progression to ESRD, and HDL cholesterol was associated with PDR. Therefore, our results support an additive effect of triglycerides and other lipid variables on microvascular complications beyond glycemic control.

Triglycerides are often a univariate predictor of CVD, but not an independent predictor when the multivariate models are adjusted with other lipid variables, which is probably due to the strong correlations between triglycerides and both non-HDL cholesterol and HDL cholesterol (335). A study from the Emerging Risk Factor Collaboration (ERFC) including 68 prospective studies, with individual data from a total of 302 430 participants, showed that triglycerides were not an independent predictor of CAD after adjustments for HDL and non-HDL cholesterol, whereas HDL cholesterol and non-HDL cholesterol remained independent predictors after the adjustments (395). In addition, there is no evidence from clinical trials that lowering triglycerides reduces CVD events after adjusting for HDL cholesterol. However, a genetic association study showed that triglycerides are a causal risk factor of CAD independently of HDL and LDL cholesterol, whereas genetic variants primarily associated with HDL cholesterol were not associated with CAD after adjustments with triglycerides and LDL cholesterol (396) (see also discussion in Section 7.4). Importantly, increased triglyceride concentrations are associated with higher remnant cholesterol concentrations (i.e. VLDL and IDL cholesterol as well as chylomicron remnants in the non-fasting state). A causal association between increased remnant cholesterol concentrations and both CAD and low-grade inflammation was shown in a Mendelian randomization study, whereas increased LDL cholesterol concentrations were causally associated with CAD, but not with inflammation (397). LDL cholesterol will remain the primary treatment target due to strong evidence from clinical trials, but increasing evidence highlights the superiority of either non-HDL cholesterol or ApoB over LDL cholesterol for CVD risk prediction (398-400). However, expert opinion is divided regarding whether or not apolipoprotein measurements should replace cholesterol measurements, and the evidence is conflicting. The ERFC Study found that non-HDL/HDL cholesterol and ApoB/ApoA-I ratios had very similar HR for CAD (395), whereas in a meta-analysis including 233 455 participants the mean relative risk ratio for ApoB was 12% higher than for LDL cholesterol and 6% higher than for non-HDL cholesterol (401). The conflicting results could be caused by differences between study populations and in the proportions of patients with metabolic dyslipidemia (i.e. high

triglycerides, low HDL cholesterol, and sdLDL cholesterol) included in the studies, and it has been suggested that ApoB may be a better CVD predictor in these patients (402). In our study, the HR of ApoB and non-HDL cholesterol for CAD events was 1.40 and 1.27, respectively, and in the NRI analyses ApoB correctly reclassified more patients than non-HDL cholesterol when it was added to the multivariate model (7.7% [p=0.01] vs. 4.7% [p=0.06]). All in all, the difference between non-HDL cholesterol and ApoB seems to be small at least in the general population, and the clear benefits of calculating non-HDL cholesterol lie in its good clinical availability and in creation of no additional costs when total cholesterol and HDL cholesterol have been measured. On the other hand, ApoB measurements have become less expensive, their clinical availability has improved, and computationally estimated ApoB with strong correlations (r=0.93-0.98) and no additional costs has been developed (403). As both non-HDL cholesterol and ApoB perform better than LDL cholesterol, the use of either one in the clinical setting should be emphasized in order to capture residual CAD risk.

7.7 Lipid profiles in patients with "double diabetes"

Importantly, in patients with renal disease, poor glycemic control, or high BMI, as seen in Study I, the lipid profile resembles that of patients with type 2 diabetes. We have previously shown that the weight-adjusted insulin dose tends to be similar or even higher in such patients (404), which suggests that the dyslipidemia is more related to increased insulin resistance than to inadequate insulin administration. The concept of "double diabetes" (i.e. when patients with type 1 diabetes exhibit features of type 2 diabetes and insulin resistance) has been proposed to describe this phenomenon (405). The prevalence of double diabetes is increasing as a consequence of increased adiposity worldwide, and the prevalence of metabolic syndrome in the FinnDiane cohort is as high as 40% in women and 38% in men (230). The triglyceride/HDL cholesterol ratio, which strongly correlates with insulin resistance (406), predicted progression to macroalbuminuria and ESRD in Study II, was associated with retinopathy in Study III, and predicted CAD events in the entire cohort, in women, in patients with normal AER, and in patients with HbA_{1c} below the median of the cohort in Study IV. These data are supported by the DCCT Study, in which eGDR, an estimate of insulin sensitivity, strongly predicted the development of nephropathy, retinopathy, and CVD (232).

7.8 Lipid thresholds and prediction

Cut-off values are widely used in the clinical setting, however, many continuous biological risk factors lack clear thresholds. This is also true for the lipid variables. In our study we could not find any clinically relevant thresholds for any of the lipid variables with regard to either micro- or macrovascular complications. However, the currently recommended cut-off level (<1.7 mmol/l) for triglycerides seems to be too high for

patients with type 1 diabetes with regard to renal disease progression and prediction of an incident CAD event. However, the cut-off of 1.7 mmol/l derives from studies for the prevention of cardiovascular disease in the general population. Thus, the cut-off for prediction of renal disease progression is unknown, but it is also noteworthy that the currently recommended cut-off level was unable to predict an incident CAD event in patients with normal AER. AER is also a continuous risk factor for micro- and macrovascular complications, and even a mild increase within the normoalbuminuric range predicts adverse outcomes (407, 408). Therefore, it has been proposed that we should cease to use the traditional categories of normo-, micro-, and macroalbuminuria and aim for an earlier risk evaluation by a multifactorial approach. Studies of renal biopsies from patients with type 1 and type 2 diabetes also support this notion (353).

7.9 Multifactorial approach

Multiple risk factors often cluster in the same patients. In Study IV, we showed that the increase in HR for incident CAD events was not linear, and an additive effect with increasing number of risk factors in patients with three or more risk factors was seen. From a practical point of view, risk calculators are needed for clinicians to be able to take into account multiple and continuous risk factors. Many calculators already exist for the prediction of CVD, but few are specifically designed for patients with type 1 diabetes, and risk calculators for prediction of renal disease are even scarcer. A multifactorial approach is also needed for the prevention of both micro- and macrovascular complications. In the Steno-2 Study, including patients with type 2 diabetes and microalbuminuria at baseline, 160 patients were randomized to receive either conventional or intensified multifactorial treatment (188). Intensified treatment included both lifestyle modifications and pharmacological treatments and reduced, for example, total cholesterol by 50 mg/dl ≈ 1.3 mmol/l, triglycerides by 41 mg/dl ≈ 0.5 mmol/l, SBP by 14 mmHg, and HbA_{1c} by 0.5%. After 7.8 years of follow-up, intensified treatment reduced the risk of CVD events by 53%, progression to macroalbuminuria by 61%, and development or progression of retinopathy by 58%. Experimental studies also support a multifactorial pharmacological approach. In rats with massive proteinuria and renal lesions, the combination of an ACE inhibitor and a statin significantly reduced glomerulosclerosis, interstitial inflammation, and tubular damage, more than the effect of either drug alone (318). However, it is quite clear that more randomized clinical trials are still needed to clarify the role of lipid variables and lipid-lowering treatment in the prevention of microvascular complications. We still lack large trials with multifactorial approaches that are initiated at an early stage of the disease process, take into account concomitant microvascular complications, and include a sufficient number of patients and sufficiently long follow-up periods.

8 SUMMARY AND CONCLUSIONS

8.1 Study I

In patients with type 1 diabetes, not only increased AER but also impaired renal function (eGFR<60 ml/min/1.73 m^2) is associated with lipid abnormalities. Changes in the lipid profile can be seen already at the stage of microalbuminuria, but are more evident in patients with macroalbuminuria. A number of patients in this study would have exceeded the internationally recommended lipid targets for the prevention of CVD, and the targets were particularly poorly met with respect to the LDL cholesterol concentrations. In patients with type 1 diabetes and impaired renal function or macroalbuminuria, the targets were especially poorly met, even though these are the patients who should be treated most aggressively. Further, patients without signs of renal disease, but with poor glycemic control, hypertension, or obesity also frequently exceeded the recommended lipid targets.

8.2 Study II

Triglycerides were an independent risk factor for the development or progression of renal disease at all stages. ApoB was also an independent predictor of progression to micro- and macroalbuminuria. Total cholesterol predicted progression from macroalbuminuria to ESRD independently of eGFR. The triglyceride concentration needed to increase the risk of progression of renal disease was much lower than the currently recommended cut-off level for triglycerides (<1.7 mmol/l), which is based on studies aimed at preventing cardiovascular disease. When ROC curves and predicted probability plots were performed, no clinically relevant thresholds emerged, but whether lower lipid targets than those currently recommended for CVD would be beneficial with regard to the progression of renal disease remains to be elucidated.

8.3 Study III

The total HDL and HDL_2 cholesterol concentrations were inversely associated with PDR independently of diabetes duration, metabolic control, blood pressure, and renal disease. The triglycerides were independently associated with mild NPDR. We observed interactions between retinopathy, nephropathy, and most lipid variables. The previously reported associations between AER and lipid variables were not seen in patients without signs of retinopathy. Furthermore, the correlations between AER and lipid variables were much stronger in patients with PDR than in patients with only mild NPDR. The results were also replicated in an independent cohort of 1100 patients with information on laser

treatment available. The results suggest the existence of shared pathological mechanisms between diabetic retinopathy and nephropathy.

8.4 Study IV

The predictive ability of lipid variables differed substantially depending on the patient's sex, renal status, and glycemic control and was substantially different from the traditional cholesterol-centric view. Total and LDL cholesterol were poor predictors of an incident CAD event in patients with normal AER, in patients with HbA_{1c} below the median of the cohort, and in women, in whom the ratios of atherogenic and anti-atherogenic lipoproteins and lipids as well as triglycerides performed better. The current guidelines may need to be revised to capture residual CAD risk in patients with type 1 diabetes.

9 ACKNOWLEDGMENTS

This study was carried out at the Folkhälsan Institute of Genetics, Folkhälsan Research Center, at the Division of Nephrology, Department of Medicine, Helsinki University Central Hospital and at Research Programs Unit, Diabetes and Obesity, University of Helsinki. I wish to acknowledge the leaders of these institutes, Professor Anna-Elina Lehesjoki, Docent Eero Honkanen and Professor Mikael Knip for providing excellent research facilities.

I am deeply grateful to my supervisor Professor Per-Henrik Groop for introducing me to the captivating world of diabetes research. I am grateful for his enthusiasm and his belief in me and all his researchers. His confidence is contagious and makes us perform better and achieve more. The uncountable hours spent editing and fine tuning the manuscripts are also highly appreciated.

I am sincerely indebted to my unofficial supervisor Carol Forsblom for his guidance throughout the process and especially during the first years when I needed it the most. He has always made time for me and without his advice, inspiring ideas and patience this work would not have been possible. Our mutual interest in music made our paths join over 15 years ago in Ostrobothnia and I am grateful for the fact that he introduced me to the FinnDiane Study Group later on when I was a medical student.

I wish to express my gratitude to Professor Marja-Riitta Taskinen for sharing her knowledge and expertise in lipid research. I am extremely grateful for her many valuable comments and her contribution to the work.

The reviewers of this thesis, Docent Jorma Lahtela and Professor Timo Strandberg are warmly acknowledged for their constructive comments and valuable suggestions. I also wish to thank Carol Ann Pelli for the help with co-editing the manuscript.

Kustaa Hietala is gratefully acknowledged for his enormous work in collecting and analyzing the fundus photographs and ophthalmic records. I want to thank Valma Harjutsalo for her kind help and advice with statistical analyses. I am also grateful to all the other co-authors Ville-Petteri Mäkinen, Daniel Gordin, Lena Thorn, Johan Wadén, Markku Saraheimo, Niina Sandholm, Maija Feodoroff, Kim Pettersson-Fernholm, Outi Heikkilä, Janne Kytö and Paula Summanen for their excellent comments and contributions.

I am grateful for being a part of the FinnDiane Study Group. I want to thank the whole "FinnDiane-family" for providing an excellent working environment. I want to thank Milla for enticing me to join FinnDiane together with Carol, and for all the singing we have done together during the years. Special thanks also goes to Jenny, Anna T, Chris, Aila, Emma, Mariann, Maikki, Nadja, Nanna, Laura, Anna-Reetta, Mari, Nanne, Anna S, Jaana and Nicu for energizing lunch and coffee breaks and all the enjoyable conference

trips. I will always fondly remember our extra “beach-holiday” in Italy after the EDNSG-meeting in Frascati in May with chillier winds than in Finland during the same time, but the weather does not matter as long as you are in good company.

I want to thank all the physicians and nurses for recruiting the patients and all the patients for giving their extra time and participating in the FinnDiane Study, thereby enabling this work. The contributions of the personnel at the lipid research laboratory of Professor Marja-Riitta Taskinen, especially Hannele Hilden and Helinä Perttunen-Nio, are also warmly acknowledged.

I am grateful to my family and all my friends for having them in my life. I could not manage without you! I specially wish to express my gratitude to my mother, who has always been there for me and supported me in difficult times, especially after my father passed away. I am also grateful for my sister Sari’s support and belief in me. Further, I want to thank my grandmother, for being like a third parent to me and taking care of me many times when I was little and got sick and for continuing to take care of me even when I was not so little anymore. I want to express my gratitude to Lauri’s family for their support and for making me feel like a part of their family. I am also grateful for my best friend from childhood, Eva, who has known me through thick and thin. Moreover, I wish to thank my former roommate and current friend Andrea for the fun years we shared while living under the same roof and for her inspiring enthusiasm. Special thanks also go to my friends from medical school, Anna-Maria, Cathina, Hannah and Lisa for their support during and after our study time together, and for our many wine and cheese tasting evenings. I also want to thank all my singing friends, for being able to make music with you.

My deepest gratitude belongs to Lauri, my fiancé and partner in life, for his love and enormous support. He has helped with corrections of my texts countless times and also showed incredible understanding when research work has sometimes interfered with our holiday plans, e.g. when I have been making suggested changes by reviewers in Turkey or correcting the proofs of a manuscript in Madeira due to deadline crises.

The work of my thesis was financially supported by Folkhälsan Research Foundation, Finnish Medical Society (Finska Läkaresällskapet), Wilhelm and Else Stockmann Foundation, Diabetes Research Foundation (Diabetestutkimussäätiö), Finnish Medical Society Duodecim, Dorothea Olivia, Karl Walter and Jarl Walter Perklen Foundation, Finnish Kidney Foundation, and Biomedicum Helsinki Foundation. They are all gratefully acknowledged.

Helsinki, December 2014

Nina Tolonen

APPENDIX

The Finnish Diabetic Nephropathy Study Centers	Physicians and nurses
Anjalankoski Health Center	S. Koivula, T. Uggeldahl
Central Finland Central Hospital, Jyväskylä	T. Forslund, A. Halonen, A. Koistinen, P. Koskiaho, M. Laukkanen, J. Saltevo, M. Tiihonen
Central Hospital of Åland Islands, Mariehamn	M. Forsen, H. Granlund, A-C. Jonsson, B. Nyroos
Central Hospital of Kanta-Häme, Hämeenlinna	P. Kinnunen, A. Orvola, T. Salonen, A. Vähänen
Central Hospital of Kymenlaakso, Kotka	R. Paldanius, M. Riihelä, L. Ryysy
Central Hospital of Länsi-Pohja, Kemi	H. Laukkanen, P. Nyländen, A. Sademies
Central Ostrabothnian Hospital District, Kokkola	S. Anderson, B. Asplund, U. Byskata, M. Kuusela, P. Liedes, T. Virkkala
City of Espoo Health Centre:	
-Espoonlahti	A. Nikkola, E. Ritola
-Samaria	E. Oukko-Ruponen, T. Virtanen
-Tapiola	M. Niska, H. Saarinen
-Viherlaakso	A. Lyytinen
City of Helsinki Health Centre:	
-Puistola	H. Kari, T. Simonen
-Suutarila	A. Kaprio, J. Kärkkäinen, B. Rantaeskola
-Töölö	J. Haaga, P. Kääriäinen, A-L. Pietiläinen
City of Hyvinkää Health Centre	S. Klemetti, T. Nyandoto, E. Rontu, S. Satuli-Autere
City of Vantaa Health Centre:	
-Korso	R. Toivonen, H. Virtanen
-Länsimäki	R. Ahonen, M. Ivaska-Suomela, A. Jauhiainen
-Martinlaakso	M. Laine, T. Pellonpää, R. Puranen
-Myyrmäki	A. Airas, J. Laakso, K. Rautavaara
-Rekola	M. Erola, E. Jatkola
-Tikkurila	R. Lönnblad, A. Malm, J. Mäkelä, E. Rautamo
Heinola Health Centre	P. Hentunen, J. Lagerstam
Helsinki University Central Hospital, Department of Medicine, Division of Nephrology	M. Feodoroff, C. Forsblom, D. Gordin, V. Harjutsalo, K. Hietala, J. Kytö, M. Parkkonen, M. Rahkonen, M. Rosengård-Bärlund, A.-R. Salonen, L. Salovaara, A. Sandelin, M. Saraheimo, T. Soppela, A. Soro-Paavonen, L. Thorn, J. Tuomikangas, J. Wadén
Herttoniemi Hospital, Helsinki	V. Sipilä

Hospital of Lounais-Häme, Forssa	T. Kalliomäki, J. Koskelainen, R. Nikkanen, N. Savolainen, H. Sulonen, E. Valtonen
Hyvinkää Hospital	A. Hämäläinen, L. Norvio
Iisalmi Hospital	E. Toivanen
Jokilaakso Hospital, Jämsä	A. Parta, I. Pirttiniemi
Jorvi Hospital, Helsinki University Central Hospital	S. Aranko, S. Ervasti, R. Kauppinen-Mäkelin, A. Kuusisto, T. Leppälä, K. Nikkilä, L. Pekkonen
Jyväskylä Health Centre, Kyllö	K. Nuorva, M. Tiihonen
Kainuu Central Hospital, Kajaani	S. Jokelainen, P. Kemppainen, A-M. Mankinen, M. Sankari
Kerava Health Centre	H. Stuckey, P. Suominen
Kirkkonummi Health Centre	A. Lappalainen, M. Liimatainen, J. Santaholma
Kivelä Hospital, Helsinki	A. Aimolahti, E. Huovinen
Koskela Hospital, Helsinki	V. Ilkka, M. Lehtimäki
Kotka Heath Centre	E. Pälikkö-Kontinen, A. Vanhanen
Kouvola Health Centre	E. Koskinen, T. Siitonen
Kuopio University Hospital	E. Huttunen, R. Ikäheimo, P. Karhapää, P. Kekäläinen, T. Lakka, M. Laakso, E. Lampainen, L. Moilanen, L. Niskanen, U. Tuovinen, I. Vauhkonen, E. Voutilainen
Kuusamo Health Centre	E. Isopoussu, T. Kääriäinen
Kuusankoski Hospital	E. Kilkki, I. Koskinen, L. Riihelä
Laakso Hospital, Helsinki	T. Meriläinen, P. Poukka, R. Savolainen, N. Uhlenius
Lahti City Hospital	A. Mäkelä, M. Tanner
Lapland Central Hospital, Rovaniemi	L. Hyvärinen, S. Severinkangas, T. Tulokas
Lappeenranta Health Centre	P. Linkola, I. Pulli
Lohja Hospital	T. Granlund, M. Saari, T. Salonen
Loimaa Health Centre	P. Eloranta, A. Mäkelä
Länsi-Uusimaa Hospital, Tammisaari	I-M. Jousmaa, J. Rinne
Malmi Hospital, Helsinki	H. Lanki, S. Moilanen, M. Tilly-Kiesi
Mikkeli Central Hospital	A. Gynther, R. Manninen, P. Nironen, M. Salminen, T. Vänttinen
Mänttä Regional Hospital	A-M. Hänninen, I. Pirttiniemi
North Karelian Hospital, Joensuu	U-M. Henttula, P. Kekäläinen, M. Pietarinen, A. Rissanen, M. Voutilainen
Nurmijärvi Health Centre	A. Burgos, K. Urtamo
Oulankangas Hospital, Oulainen	E. Jokelainen, P-L. Jylkkä, E. Kaarlela, J. Vuolaspuro
Oulu Health Centre	L. Hiltunen, R. Häkkinen, S. Keinänen-Kiukaanniemi
Oulu University Hospital	R. Ikäheimo
Päijät-Häme Central Hospital	H. Haapamäki, A. Helanterä, S. Hämäläinen, V. Ilvesmäki, H. Miettinen

Palokka Health Centre	P. Sopanen, L. Welling
Pieksämäki Hospital	V. Javtsenko, M. Tamminen
Pietarsaari Hospital	M-L. Holmbäck, B. Isomaa, L. Sarelin
Pori City Hospital	P. Ahonen, P. Merensalo, K. Sävelä
Porvoo Hospital	M. Kallio, B. Rask, S. Rämö
Raahe Hospital	A. Holma, M. Honkala, A. Tuomivaara, R. Vainionpää
Rauma Hospital	K. Laine, K. Saarinen, T. Salminen
Riihimäki Hospital	P. Aalto, E. Immonen, L. Juurinen
Salo Hospital	A. Alanko, J. Lapinleimu, P. Rautio, M. Virtanen
Satakunta Central Hospital, Pori	M. Asola, M. Juhola, P. Kunelius, M-L. Lahdenmäki, P. Pääkkönen, M. Rautavirta
Savonlinna Central Hospital	E. Korpi-Hyövälti, T. Latvala, E. Leijala
South Karelia Central Hospital, Lappeenranta	T. Ensala, E. Hussi, R. Härkönen, U. Nyholm, J. Toivanen
Tampere Health Centre	P. Alarotu, L. Calonius, S. Gummerus , M. Helin, T. Kaitala, H. Kirkkopelto-Jokinen, E. Kujansuu, T. Niskanen, A. Vaden, T. Vatanen
Tampere University Hospital	I. Ala-Houhala, T. Kuningas, P. Lampinen, M. Määttä, H. Oksala, T. Oksanen, K. Salonen, H. Tauriainen, S. Tulokas
Tiirismaa Health Centre, Hollola	T. Kivelä, L, Petlin, L. Savolainen
Turku Health Centre	I. Hämäläinen, H. Virtamo, M. Vähätalo
Turku University Central Hospital	K. Breitholz, R. Eskola, K. Metsärinne, U. Pietilä, P. Saarinen, R. Tuominen, S. Äyräpää
Vaajakoski Health Centre	K. Mäkinen, P. Sopanen
Vaasa Central Hospital	S. Bergkulla, U. Hautamäki, V-A. Myllyniemi, I. Rusk
Valkeakoski Regional Hospital	T. Immonen, S. Ojanen, M. Rautiainen, E. Valtonen, H. Ylönen
Vammala Regional Hospital	I. Isomäki, R. Kroneld, M. Tapiolinna-Mäkelä

10 REFERENCES

1. Laing S, Swerdlow A, Slater S, Botha J, Burden A, Waugh N, Smith A, Hill R, Bingley P, Patterson C: The British Diabetic Association Cohort Study, II: cause-specific mortality in patients with insulin-treated diabetes mellitus. *Diabetic Med* 16:466-471, 1999
2. Groop PH, Thomas MC, Moran JL, Wadén J, Thorn LM, Mäkinen VP, Rosengård-Bärlund M, Saraheimo M, Hietala K, Heikkilä O, Forsblom C, FinnDiane Study Group: The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. *Diabetes* 58:1651-1658, 2009
3. Orchard T, Secrest A, Miller R, Costacou T: In the absence of renal disease, 20 year mortality risk in type 1 diabetes is comparable to that of the general population: a report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia* 53:2312-2319, 2010
4. Klein R, Klein BEK: Vision disorders in diabetes. *Diabetes in America* 2:293-338, 1995
5. Guariguata L, Whiting D, Hambleton I, Beagley J, Linnenkamp U, Shaw J: Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 103:137-149, 2014
6. Sund R, Koski S: FinDM II. On the register-based measurement of the prevalence and incidence of diabetes and its long-term complications. *Finnish Diabetes Association* p.1-24, 2010
7. Harjutsalo V, Sjöberg L, Tuomilehto J: Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *The Lancet* 371:1777-1782, 2008
8. Ilanne-Parikka P: Tyypin 1 diabetesta sairastavien hoidon kehittäminen, asiantuntijaryhmän raportti. *Finnish Diabetes Association* p.1-17, 2014
9. Onkamo P, Väänänen S, Karvonen M, Tuomilehto J: Worldwide increase in incidence of Type I diabetes–the analysis of the data on published incidence trends. *Diabetologia* 42:1395-1403, 1999
10. Lithovius R, Harjutsalo V, Forsblom C, Saraheimo M, Groop PH: The consequences of failure to achieve targets of guidelines for prevention and treatment of diabetic complications in patients with type 1 diabetes. *Acta Diabetol* (Epub ahead of print) doi: 10.1007/s00592-014-0595-x, 2014
11. Jönsson B: Revealing the cost of type II diabetes in Europe. *Diabetologia* 45:S5-S12, 2002
12. Kramer A, Stel V, Zoccali C, Heaf J, Ansell D, Grönhagen-Riska C, Leivestad T, Simpson K, Palsson R, Postorino M, Jager K, ERA-EDTA Registry: An update on renal replacement therapy in Europe: ERA-EDTA Registry data from 1997 to 2006. *Nephrol Dial Transplant* 24:3557-3566, 2009
13. Hovind P, Tarnow L, Rossing P, Graae M, Torp I, Binder C, Parving HH: Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study. *BMJ* 328:1105-1109, 2004
14. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in type 1 (insulin-dependent) diabetes: an epidemiological study. *Diabetologia* 25:496-501, 1983
15. Klein R, Knudtson MD, Lee KE, Gangnon R, Klein BEK: The Wisconsin Epidemiologic Study of Diabetic Retinopathy XXII: The Twenty-Five-Year Progression of Retinopathy in Persons with Type 1 Diabetes. *Ophthalmology* 115:1859-1868, 2008
16. Klein R: The epidemiology of diabetic retinopathy: Findings from the Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Int Ophthalmol Clin* 27:230-238, 1987
17. Kostraba JN, Klein R, Dorman JS, Becker DJ, Drash AL, Maser RE, Orchard TJ: The Epidemiology of Diabetes Complications Study: IV. Correlates of diabetic background and proliferative retinopathy. *Am J Epidemiol* 133:381-391, 1991

18. Marshall G, Garg SK, Jackson WE, Holmes DL, Chase HP: Factors influencing the onset and progression of diabetic retinopathy in subjects with insulin-dependent diabetes mellitus. *Ophthalmology* 100:1133-1139, 1993
19. Agardh CD, Agardh E, Bauer B, Nilsson-Ehle P: Plasma lipids and plasma lipoproteins in diabetics with and without proliferative retinopathy. *Acta Med Scand* 223:165-169, 1988
20. Sjølie AK, Stephenson J, Aldington S, Kohner E, Janka H, Stevens L, Fuller J: Retinopathy and vision loss in insulin-dependent diabetes in Europe: the EURODIAB IDDM Complications Study. *Ophthalmology* 104:252-260, 1997
21. Libby P, Nathan DM, Abraham K, Brunzell JD, Fradkin JE, Haffner SM, Hsueh W, Rewers M, Roberts BT, Savage PJ, Skarlatos S, Wassef M, Rabadan-Diehl C, National Heart, Lung, and Blood Institute, National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus: Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. *Circulation* 111:3489-3493, 2005
22. World Health Organization: Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. p.1-46, 2006
23. World Health Organization: Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. p.1-25, 2011
24. American Diabetes Association: Diagnosis and classification of diabetes mellitus. *Diabetes Care* 37:S81-S90, 2014
25. Wilkin T: The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. *Diabetologia* 44:914-922, 2001
26. Pozzilli P, Di Mario U: Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. *Diabetes Care* 24:1460-1467, 2001
27. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR: Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes* 42:359-362, 1993
28. Fajans SS, Bell GI, Polonsky KS: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345:971-980, 2001
29. Kim C, Newton KM, Knopp RH: Gestational diabetes and the incidence of type 2 diabetes: a systematic review. *Diabetes Care* 25:1862-1868, 2002
30. Rehman A, Setter SM, Vue MH: Drug-induced glucose alterations part 2: drug-induced hyperglycemia. *Diabetes Spectrum* 24:234-238, 2011
31. Preiss D, Seshasai SRK, Welsh P, Murphy SA, Ho JE, Waters DD, DeMicco DA, Barter P, Cannon CP, Sabatine MS: Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis. *JAMA* 305:2556-2564, 2011
32. Östman J, Lönnberg G, Arnqvist H, Blohme G, Bolinder J, Schnell AE, Eriksson J, Gudbjörnsdottir S, Sundkvist G, Nyström L: Gender differences and temporal variation in the incidence of type 1 diabetes: results of 8012 cases in the nationwide Diabetes Incidence Study in Sweden 1983–2002. *J Intern Med* 263:386-394, 2008
33. The DIAMOND Project Group: Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. *Diabetic Med* 23:857-866, 2006
34. Harjutsalo V, Sund R, Knip M, Groop PH: Incidence of type 1 diabetes in Finland. *JAMA* 310:427-428, 2013
35. Berhan Y, Waernbaum I, Lind T, Mollsten A, Dahlquist G, Swedish Childhood Diabetes Study Group: Thirty years of prospective nationwide incidence of childhood type 1 diabetes: the accelerating increase by time tends to level off in Sweden. *Diabetes* 60:577-581, 2011

36. Karvonen M, Pitkäniemi J, Tuomilehto J: The onset age of type 1 diabetes in Finnish children has become younger. The Finnish Childhood Diabetes Registry Group. *Diabetes Care* 22:1066-1070, 1999
37. Pundziute-Lyckå A, Dahlquist G, Nyström L, Arnqvist H, Björk E, Blohme G, Bolinder J, Eriksson J, Sundkvist G, Östman J: The incidence of type I diabetes has not increased but shifted to a younger age at diagnosis in the 0–34 years group in Sweden 1983 to 1998. *Diabetologia* 45:783-791, 2002
38. Gale EA: Spring harvest? Reflections on the rise of type 1 diabetes. *Diabetologia* 48:2445-2450, 2005
39. Weets I, De Leeuw IH, Du Caju MV, Rooman R, Keymeulen B, Mathieu C, Rottiers R, Daubresse JC, Rocour-Brumioul D, Pipeleers DG, Gorus FK, Belgian Diabetes Registry: The incidence of type 1 diabetes in the age group 0-39 years has not increased in Antwerp (Belgium) between 1989 and 2000: evidence for earlier disease manifestation. *Diabetes Care* 25:840-846, 2002
40. Lammi N, Blomstedt P, Moltchanova E, Eriksson J, Tuomilehto J, Karvonen M: Marked temporal increase in the incidence of type 1 and type 2 diabetes among young adults in Finland. *Diabetologia* 51:897-899, 2008
41. Feltbower R, Bodansky H, McKinney P, Houghton J, Stephenson C, Haigh D: Trends in the incidence of childhood diabetes in south Asians and other children in Bradford, UK. *Diabetic Med* 19:162-166, 2002
42. Oilinki T, Otonkoski T, Ilonen J, Knip M, Miettinen P: Prevalence and characteristics of diabetes among Somali children and adolescents living in Helsinki, Finland. *Pediatric diabetes* 13:176-180, 2012
43. Willcox A, Richardson S, Bone A, Foulis A, Morgan N: Analysis of islet inflammation in human type 1 diabetes. *Clinical & Experimental Immunology* 155:173-181, 2009
44. Ziegler A, Nepom GT: Prediction and pathogenesis in type 1 diabetes. *Immunity* 32:468-478, 2010
45. Knip M: Natural course of preclinical type 1 diabetes. *Horm Res* 57: Suppl 1:6-11, 2002
46. Concannon P, Rich SS, Nepom GT: Genetics of type 1A diabetes. *N Engl J Med* 360:1646-1654, 2009
47. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C: Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 41:703-707, 2009
48. Noble JA, Valdes AM, Varney MD, Carlson JA, Moonsamy P, Fear AL, Lane JA, Lavant E, Rappner R, Louey A, Concannon P, Mychaleckyj JC, Erlich HA, Type 1 Diabetes Genetics Consortium: HLA class I and genetic susceptibility to type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium. *Diabetes* 59:2972-2979, 2010
49. Hermann R, Knip M, Veijola R, Simell O, Laine A, Åkerblom H, Groop P, Forsblom C, Pettersson-Fernholm K, Ilonen J: Temporal changes in the frequencies of HLA genotypes in patients with Type 1 diabetes—indication of an increased environmental pressure? *Diabetologia* 46:420-425, 2003
50. Scott FW: Cow milk and insulin-dependent diabetes mellitus: is there a relationship? *Am J Clin Nutr* 51:489-491, 1990
51. Myers MA, Mackay IR, Zimmet PZ: Toxic type 1 diabetes. *Reviews in Endocrine and Metabolic Disorders* 4:225-231, 2003
52. Cronin CC, Shanahan F: Insulin-dependent diabetes mellitus and coeliac disease. *The Lancet* 349:1096-1097, 1997
53. Borch-Johnsen K, Mandrup-Poulsen T, Zachau-Christiansen B, Joner G, Christy M, Kastrup K, Nerup J: Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus: a hypothesis. *The Lancet* 324:1083-1086, 1984

54. Cooper JD, Smyth DJ, Walker NM, Stevens H, Burren OS, Wallace C, Greissl C, Ramos-Lopez E, Hypponen E, Dunger DB, Spector TD, Ouwehand WH, Wang TJ, Badenhoop K, Todd JA: Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. *Diabetes* 60:1624-1631, 2011
55. Janner M, Ballinari P, Mullis P, Fluck C: High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. *Swiss Med Wkly* 140:w13091, 2010
56. Craig ME, Nair S, Stein H, Rawlinson WD: Viruses and type 1 diabetes: a new look at an old story. *Pediatric Diabetes* 14:149-158, 2013
57. Cardwell C, Stene L, Joner G, Cinek O, Svensson J, Goldacre M, Parslow R, Pozzilli P, Brigis G, Stoyanov D: Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. *Diabetologia* 51:726-735, 2008
58. Boerner BP, Sarvetnick NE: Type 1 diabetes: role of intestinal microbiome in humans and mice. *Ann N Y Acad Sci* 1243:103-118, 2011
59. Kahn HS, Morgan TM, Case LD, Dabelea D, Mayer-Davis EJ, Lawrence JM, Marcovina SM, Imperatore G, SEARCH for Diabetes in Youth Study Group: Association of type 1 diabetes with month of birth among U.S. youth: The SEARCH for Diabetes in Youth Study. *Diabetes Care* 32:2010-2015, 2009
60. Moltchanova E, Schreier N, Lammi N, Karvonen M: Seasonal variation of diagnosis of Type 1 diabetes mellitus in children worldwide. *Diabetic Med* 26:673-678, 2009
61. Bach JF, Chatenoud L: The hygiene hypothesis: an explanation for the increased frequency of insulin-dependent diabetes. *Cold Spring Harb Perspect Med* 4:a007799, 2012
62. Tedeschi A, Airaghi L: Is affluence a risk factor for bronchial asthma and type 1 diabetes? *Pediatric Allergy and Immunology* 17:533-537, 2006
63. Patterson C, Carson D, Hadden D: Epidemiology of childhood IDDM in Northern Ireland 1989–1994: low incidence in areas with highest population density and most household crowding. *Diabetologia* 39:1063-1069, 1996
64. McKinney P, Okasha M, Parslow R, Law G, Gurney K, Williams R, Bodansky H: Early social mixing and childhood type 1 diabetes mellitus: a case–control study in Yorkshire, UK. *Diabetic Med* 17:236-242, 2000
65. Oresic M, Simell S, Sysi-Aho M, Näntö-Salonen K, Seppänen-Laakso T, Parikka V, Katajamaa M, Hekkala A, Mattila I, Keskinen P, Yetukuri L, Reinikainen A, Lähde J, Suortti T, Hakalax J, Simell T, Hyöty H, Veijola R, Ilonen J, Lahesmaa R, Knip M, Simell O: Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes. *J Exp Med* 205:2975-2984, 2008
66. Pflueger M, Seppänen-Laakso T, Suortti T, Hyötyläinen T, Achenbach P, Bonifacio E, Oresic M, Ziegler AG: Age- and islet autoimmunity-associated differences in amino acid and lipid metabolites in children at risk for type 1 diabetes. *Diabetes* 60:2740-2747, 2011
67. Mogensen CE, Vestbo E, Poulsen PL, Christiansen C, Damsgaard EM, Eiskjaer H, Froland A, Hansen KW, Nielsen S, Pedersen MM: Microalbuminuria and potential confounders. A review and some observations on variability of urinary albumin excretion. *Diabetes Care* 18:572-581, 1995
68. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL, Eckardt K: The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int* 80:17-28, 2010
69. Bröchner-Mortensen J: A simple method for the determination of glomerular filtration rate. *Scandinavian Journal of Clinical & Laboratory Investigation* 30:271-274, 1972
70. Bröchner-Mortensen J, Giese J, Rossing N: Renal inulin clearance versus total plasma clearance of 51Cr-EDTA. *Scandinavian Journal of Clinical & Laboratory Investigation* 23:301-305, 1969
71. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31-41, 1976

72. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 130:461-470, 1999
73. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T: A new equation to estimate glomerular filtration rate. *Ann Intern Med* 150:604-612, 2009
74. Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindstrom V, Grubb A: Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem* 40:1921-1926, 1994
75. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL: Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 367:20-29, 2012
76. Krolewski AS, Warram JH, Forsblom C, Smiles AM, Thorn L, Skupien J, Harjutsalo V, Stanton R, Eckfeldt JH, Inker LA, Groop PH: Serum concentration of cystatin C and risk of end-stage renal disease in diabetes. *Diabetes Care* 35:2311-2316, 2012
77. Stephenson J, Kenny S, Stevens L, Fuller J, Lee E: Proteinuria and mortality in diabetes: the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetic Med* 12:149-155, 1995
78. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in Type 1 (insulin-dependent) diabetes: an epidemiological study. *Diabetologia* 25:496-501, 1983
79. Bojestig M, Arnqvist HJ, Hermansson G, Karlberg BE, Ludvigsson J: Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med* 330:15-18, 1994
80. Forsblom CM, Groop PH, Ekstrand A, Groop LC: Predictive value of microalbuminuria in patients with insulin-dependent diabetes of long duration. *BMJ* 305:1051-1053, 1992
81. Viberti G, Jarrett R, Mahmud U, Hill R, Argyropoulos A, Keen H: Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *The Lancet* 319:1430-1432, 1982
82. Parving HH, Oxenboll B, Svendsen PA, Christiansen JS, Andersen AR: Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. *Acta Endocrinol (Copenh)* 100:550-555, 1982
83. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS: Regression of microalbuminuria in type 1 diabetes. *N Engl J Med* 348:2285-2293, 2003
84. Perkins BA, Ficociello LH, Ostrander BE, Silva KH, Weinberg J, Warram JH, Krolewski AS: Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. *J Am Soc Nephrol* 18:1353-1361, 2007
85. Parving H, Andersen AR, Smidt UM, Christiansen JS, Oxenbøll B, Svendsen PA: Diabetic nephropathy and arterial hypertension: the effect of antihypertensive treatment. *Diabetes* 32:83-87, 1983
86. Mogensen CE: Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *BMJ* 285:685-688, 1982
87. Finne P, Reunanen A, Stenman S, Groop PH, Grönhagen-Riska C: Incidence of end-stage renal disease in patients with type 1 diabetes. *JAMA* 294:1782-1787, 2005
88. Molitch ME, Steffes M, Sun W, Rutledge B, Cleary P, de Boer IH, Zinman B, Lachin J, EDIC Study Group: Development and progression of renal insufficiency with and without albuminuria in adults with type 1 diabetes in the Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications Study. *Diabetes Care* 33:1536-1543, 2010
89. Mauer SM, Steffes MW, Brown DM: The kidney in diabetes. *Am J Med* 70:603-612, 1981

90. Østeeby R: Morphometric studies of the peripheral glomerular basement membrane in early juvenile diabetes I. Development of initial basement membrane thickening. *Diabetologia* 8:84-92, 1972
91. Fioretto P, Mauer M: Histopathology of diabetic nephropathy. 27:195-207, 2007
92. Kimmelstiel P, Wilson C: Intercapillary Lesions in the Glomeruli of the Kidney. *Am J Pathol* 12:83-98, 1936
93. Kim Y, Kleppel MM, Butkowski R, Mauer SM, Wieslander J, Michael AF: Differential expression of basement membrane collagen chains in diabetic nephropathy. *Am J Pathol* 138:413-420, 1991
94. Falk RJ, Scheinman JI, Mauer SM, Michael AF: Polyantigenic expansion of basement membrane constituents in diabetic nephropathy. *Diabetes* 32:34-39, 1983
95. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, Ferrario F, Fogo AB, Haas M, de Heer E, Joh K, Noel LH, Radhakrishnan J, Seshan SV, Bajema IM, Bruijn JA, Renal Pathology Society: Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol* 21:556-563, 2010
96. Harris RD, Steffes MW, Bilous RW, Sutherland DE, Mauer SM: Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. *Kidney Int* 40:107-114, 1991
97. Najafian B, Kim Y, Crosson JT, Mauer M: Atubular glomeruli and glomerulotubular junction abnormalities in diabetic nephropathy. *J Am Soc Nephrol* 14:908-917, 2003
98. Forbes JM, Cooper ME: Mechanisms of diabetic complications. *Physiol Rev* 93:137-188, 2013
99. Stephenson J, Fuller JH: Microvascular and acute complications in IDDM patients: the EURODIAB IDDM Complications Study. *Diabetologia* 37:278-285, 1994
100. Olsen BS, Sjølie A, Hougaard P, Johannesen J, Borch-Johnsen K, Marinelli K, Thorsteinsson B, Pramming S, Mortensen HB: A 6-year nationwide cohort study of glycaemic control in young people with type 1 diabetes: risk markers for the development of retinopathy, nephropathy and neuropathy. *J Diabetes Complications* 14:295-300, 2000
101. Rossing P, Hougaard P, Parving HH: Progression of microalbuminuria in type 1 diabetes: ten-year prospective observational study. *Kidney Int* 68:1446-1450, 2005
102. Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. The Diabetes Control and Complications (DCCT) Research Group. *Kidney Int* 47:1703-1720, 1995
103. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group, Nathan DM, Zinman B, Cleary PA, Backlund JY, Genuth S, Miller R, Orchard TJ: Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications and Pittsburgh Epidemiology of Diabetes Complications Experience (1983-2005). *Arch Intern Med* 169:1307-1316, 2009
104. Turner R, Holman R, Cull C, Stratton I, Matthews D, Frighi V, Manley S, Neil A, Mcelroy K, Wright D: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837-853, 1998
105. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D: Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. 358:2560-2572, 2008
106. Abraira C, Colwell JA, Nuttall FQ, Sawin CT, Nagel NJ, Comstock JP, Emanuele NV, Levin SR, Henderson W, Lee HS: Veterans Affairs Cooperative Study on glycemic control and complications in type II diabetes (VA CSDM). Results of the feasibility trial. Veterans Affairs Cooperative Study in Type II Diabetes. *Diabetes Care* 18:1113-1123, 1995

107. Ismail-Beigi F, Craven T, Banerji MA, Basile J, Calles J, Cohen RM, Cuddihy R, Cushman WC, Genuth S, Grimm Jr RH: Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. *The Lancet* 376:419-430, 2010
108. Kilpatrick ES, Rigby AS, Atkin SL: A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial. *Diabetes Care* 31:2198-2202, 2008
109. Wadén J, Forsblom C, Thorn LM, Gordin D, Saraheimo M, Groop PH: A1C variability predicts incident cardiovascular events, microalbuminuria, and overt diabetic nephropathy in patients with type 1 diabetes. *Diabetes* 58:2649-2655, 2009
110. Hasslacher CH, Stech W, Wahl P, Ritz E: Blood pressure and metabolic control as risk factors for nephropathy in type 1 (insulin-dependent) diabetes. *Diabetologia* 28:6-11, 1985
111. Mathiesen ER, Rønn B, Jensen T, Storm B, Deckert T: Relationship between blood pressure and urinary albumin excretion in development of microalbuminuria. *Diabetes* 39:245-249, 1990
112. Hovind P, Rossing P, Tarnow L, Smidt UM, Parving H: Progression of diabetic nephropathy. *Kidney Int* 59:702-709, 2001
113. Kasiske BL, Kalil RS, Ma JZ, Liao M, Keane WF: Effect of antihypertensive therapy on the kidney in patients with diabetes: a meta-regression analysis. *Ann Intern Med* 118:129-138, 1993
114. Keane WF, Brenner BM, De Zeeuw D, Grunfeld J, Mcgill J, Mitch WE, Ribeiro AB, Shahinfar S, Simpson RL, Snapinn SM: The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. *Kidney Int* 63:1499-1507, 2003
115. Cheng J, Zhang W, Zhang X, Han F, Li X, He X, Li Q, Chen J: Effect of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on all-cause mortality, cardiovascular deaths, and cardiovascular events in patients with diabetes mellitus: a meta-analysis. *JAMA internal medicine* 174:773-785, 2014
116. American Diabetes Association: Standards of Medical Care in Diabetes-2007. *Diabetes Care* 30: Suppl 1:S4-S41, 2007
117. American Diabetes Association: Standards of medical care in diabetes-2014. *Diabetes Care* 37: Suppl 1:S14-S80, 2014
118. ACCORD Study Group, Cushman WC, Evans GW, Byington RP, Goff DC,Jr, Grimm RH,Jr, Cutler JA, Simons-Morton DG, Basile JN, Corson MA, Probstfield JL, Katz L, Peterson KA, Friedewald WT, Buse JB, Bigger JT, Gerstein HC, Ismail-Beigi F: Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med* 362:1575-1585, 2010
119. Bangalore S, Kumar S, Lobach I, Messerli FH: Blood pressure targets in subjects with type 2 diabetes mellitus/impaired fasting glucose: observations from traditional and bayesian random-effects meta-analyses of randomized trials. *Circulation* 123:2799-2810, 2011
120. DeFronzo R, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23:313-319, 1982
121. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214-E223, 1979
122. Ekstrand AV, Groop PH, Grönhagen-Riska C: Insulin resistance precedes microalbuminuria in patients with insulin-dependent diabetes mellitus. *Nephrol Dial Transplant* 13:3079-3083, 1998
123. Svensson M, Eriksson JW: No direct link between albumin excretion rate and insulin resistance - a study in type 1 diabetes patients with mild nephropathy. *Hormone and Metabolic Research* 34:254-259, 2002

124. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ: Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 49:626-632, 2000
125. Orchard TJ, Chang YF, Ferrell RE, Petro N, Ellis DE: Nephropathy in type 1 diabetes: a manifestation of insulin resistance and multiple genetic susceptibilities? Further evidence from the Pittsburgh Epidemiology of Diabetes Complication Study. *Kidney Int* 62:963-970, 2002
126. Thorn LM, Forsblom C, Wadén J, Saraheimo M, Tolonen N, Hietala K, Groop PH, on behalf of the FinnDiane Study Group: The metabolic syndrome as a risk factor for cardiovascular disease, mortality, and progression of diabetic nephropathy in type 1 diabetes. *Diabetes Care* 32:950-952, 2009
127. Fagerudd J, Pettersson-Fernholm KJ, Grönhagen-Riska C, Groop P: The impact of a family history of type II (non-insulin-dependent) diabetes mellitus on the risk of diabetic nephropathy in patients with type I (insulin-dependent) diabetes mellitus. *Diabetologia* 42:519-526, 1999
128. Quinn M, Angelico M, Warram J, Krolewski A: Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia* 39:940-945, 1996
129. Nelson R, Newman J, Knowler W, Sievers M, Kunzelman C, Pettitt D, Moffett C, Teutsch S, Bennett P: Incidence of end-stage renal disease in type 2 (non-insulin-dependent) diabetes mellitus in Pima Indians. *Diabetologia* 31:730-736, 1988
130. MacIsaac RJ, Jerums G, Watts GF: Diabetic chronic kidney disease. In *Diabetes Chronic Complications*. John Wiley & Sons, 2012, p.34-66
131. Sandholm N, Salem RM, McKnight AJ, Brennan EP, Forsblom C, Isakova T, McKay GJ, Williams WW, Sadlier DM, Mäkinen VP, Swan EJ, Palmer C, Boright AP, Ahlqvist E, Deshmukh HA, Keller BJ, Huang H, Ahola AJ, Fagerholm E, Gordin D, Harjutsalo V, He B, Heikkilä O, Hietala K, Kytö J, Lahermo P, Lehto M, Lithovius R, Österholm AM, Parkkonen M, Pitkäniemi J, Rosengård-Bärlund M, Saraheimo M, Sarti C, Söderlund J, Soro-Paavonen A, Syreeni A, Thorn LM, Tikkanen H, Tolonen N, Tryggvason K, Tuomilehto J, Wadén J, Gill GV, Prior S, Guiducci C, Mirel DB, Taylor A, Hosseini SM, DCCT/EDIC Research Group, Parving HH, Rossing P, Tarnow L, Ladenvall C, Alhenc-Gelas F, Lefebvre P, Rigalleau V, Roussel R, Tregouet DA, Maestroni A, Maestroni S, Falhammar H, Gu T, Mollsten A, Cimponeriu D, Ioana M, Mota M, Mota E, Serafinceanu C, Stavarachi M, Hanson RL, Nelson RG, Kretzler M, Colhoun HM, Panduru NM, Gu HF, Brismar K, Zerbini G, Hadjadj S, Marre M, Groop L, Lajer M, Bull SB, Waggott D, Paterson AD, Savage DA, Bain SC, Martin F, Hirschhorn JN, Godson C, Florez JC, Groop PH, & Maxwell AP: New susceptibility loci associated with kidney disease in type 1 diabetes. *PLoS Genetics* 8:e1002921, 2012
132. Veikkolainen V, Naillat F, Railo A, Chi L, Manninen A, Hohenstein P, Hastie N, Vainio S, Elenius K: ERBB4 modulates tubular cell polarity and lumen diameter during kidney development. *J Am Soc Nephrol* 23:112-122, 2012
133. Biesenbach G, Grafinger P, Janko O, Zazgornik J: Influence of cigarette-smoking on the progression of clinical diabetic nephropathy in type 2 diabetic patients. *Clin Nephrol* 48:146-150, 1997
134. Coonrod BA, Ellis D, Becker DJ, Bunker CH, Kelsey SF, Lloyd CE, Drash AL, Kuller LH, Orchard TJ: Predictors of microalbuminuria in individuals with IDDM. Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Care* 16:1376-1383, 1993
135. Thomas MC, MacIsaac RJ, Tsalamandris C, Molyneaux L, Goubina I, Fulcher G, Yue D, Jerums G: Anemia in patients with type 1 diabetes. *The Journal of Clinical Endocrinology & Metabolism* 89:4359-4363, 2004
136. Mohanram A, Zhang Z, Shahinfar S, Keane WF, Brenner BM, Toto RD: Anemia and end-stage renal disease in patients with type 2 diabetes and nephropathy. *Kidney Int* 66:1131-1138, 2004

137. Vikse BE, Irgens LM, Leivestad T, Hallan S, Iversen BM: Low birth weight increases risk for end-stage renal disease. *J Am Soc Nephrol* 19:151-157, 2008
138. Rossing P, Tarnow L, Nielsen FS, Boelskifte S, Brenner BM, Parving HH: Short stature and diabetic nephropathy. *BMJ* 310:296-297, 1995
139. Pedrini MT, Levey AS, Lau J, Chalmers TC, Wang PH: The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: A meta-analysis. *Ann Intern Med* 124:627-632, 1996
140. Saraheimo M, Forsblom C, Thorn L, Wadén J, Rosengård-Bärlund M, Heikkilä O, Hietala K, Gordin D, Frystyk J, Flyvbjerg A, Groop PH, FinnDiane Study Group: Serum adiponectin and progression of diabetic nephropathy in patients with type 1 diabetes. *Diabetes Care* 31:1165-1169, 2008
141. Beisswenger PJ, Howell SK, Russell GB, Miller ME, Rich SS, Mauer M: Early progression of diabetic nephropathy correlates with methylglyoxal-derived advanced glycation end products. *Diabetes Care* 36:3234-3239, 2013
142. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH: Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients. *Diabetologia* 46:1402-1407, 2003
143. Idzior-Walus B, Mattock MB, Solnica B, Stevens L, Fuller JH, EURODIAB IDDM Complications Study G: Factors associated with plasma lipids and lipoproteins in type 1 diabetes mellitus: the EURODIAB IDDM Complications Study. *Diabetic Med* 18:786-796, 2001
144. Jenkins AJ. Lyons TJ. Zheng D. Otvos JD. Lackland DT. McGee D. Garvey WT. Klein RL. DCCT/EDIC Research Group: Serum lipoproteins in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications cohort: associations with gender and glycemia. *Diabetes Care* 26:810-818, 2003
145. Jones SL, Close CF, Mattock MB, Jarrett RJ, Keen H, Viberti GC: Plasma lipid and coagulation factor concentrations in insulin dependent diabetics with microalbuminuria. *BMJ* 298:487-490, 1989
146. Mäkinen V, Tolonen N, Groop PH: Lipoproteins and diabetic nephropathy. In *Lipoproteins in Diabetes Mellitus.* Springer, 2014, p.279-299
147. Early Treatment Diabetic Retinopathy Study design and baseline patient characteristics. ETDRS report number 7. *Ophthalmology* 98:741-756, 1991
148. Wilkinson C, Ferris III FL, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdaguer JT: Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology* 110:1677-1682, 2003
149. Bhagat N, Grigorian RA, Tutela A, Zarbin MA: Diabetic macular edema: pathogenesis and treatment. *Surv Ophthalmol* 54:1-32, 2009
150. Keech AC, Mitchell P, Summanen PA, O'Day J, Davis TME, Moffitt MS, Taskinen MR, Simes RJ, Tse D, Williamson E: Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD Study): a randomised controlled trial. *The Lancet* 370:1687-1697, 2007
151. Skrivarhaug T, Fosmark D, Stene L, Bangstad H, Sandvik L, Hanssen K, Joner G: Low cumulative incidence of proliferative retinopathy in childhood-onset type 1 diabetes: a 24-year follow-up study. *Diabetologia* 49:2281-2290, 2006
152. Henricsson M, Nilsson A, Groop L, Heijl A, Janzon L: Prevalence of diabetic retinopathy in relation to age at onset of the diabetes, treatment, duration and glycemic control. *Acta Ophthalmol Scand* 74:523-527, 1996
153. Williams R, Airey M, Baxter H, Forrester J, Kennedy-Martin T, Girach A: Epidemiology of diabetic retinopathy and macular oedema: a systematic review. *Eye* 18:963-983, 2004

154. Wong TY, Mwamburi M, Klein R, Larsen M, Flynn H, Hernandez-Medina M, Ranganathan G, Wirostko B, Pleil A, Mitchell P: Rates of progression in diabetic retinopathy during different time periods: a systematic review and meta-analysis. *Diabetes Care* 32:2307-2313, 2009
155. Kytö JP, Harjutsalo V, Forsblom C, Hietala K, Summanen PA, Groop PH, FinnDiane Study Group: Decline in the cumulative incidence of severe diabetic retinopathy in patients with type 1 diabetes. *Diabetes Care* 34:2005-2007, 2011
156. Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings, DRS Report Number 8. *Ophthalmology* 88:583-600, 1981
157. Pirart J: Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. *Diabetes Care* 1:168-188, 1978
158. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993
159. The Diabetes Control and Complications Trial Research Group: The effect of intensive diabetes treatment on the progression of diabetic retinopathy in insulin-dependent diabetes mellitus. *Arch Ophthalmol* 113:36-51, 1995
160. Dahl-Jorgensen K, Brinchmann-Hansen O, Hanssen KF, Sandvik L, Aagenaes O: Rapid tightening of blood glucose control leads to transient deterioration of retinopathy in insulin dependent diabetes mellitus: The Oslo Study. *BMJ* 290:811-815, 1985
161. Hietala K, Wadén J, Forsblom C, Harjutsalo V, Kytö J, Summanen P, Groop PH, on behalf of the FinnDiane Study Group: HbA(1c) variability is associated with an increased risk of retinopathy requiring laser treatment in type 1 diabetes. *Diabetologia* 56:737-745, 2013
162. Klein R, Klein BE, Moss SE, Cruickshanks KJ: The Wisconsin Epidemiologic Study of Diabetic Retinopathy: XVII: The 14-year incidence and progression of diabetic retinopathy and associated risk factors in type 1 diabetes. *Ophthalmology* 105:1801-1815, 1998
163. Matthews DR, Stratton IM, Aldington SJ, Holman RR, Kohner EM, UK Prospective Diabetes Study Group: Risks of progression of retinopathy and vision loss related to tight blood pressure control in type 2 diabetes mellitus: UKPDS 69. *Arch Ophthalmol* 122:1631-1640, 2004
164. Chaturvedi N, Sjolie AK, Stephenson JM, Abrahamian H, Keipes M, Castellarin A, Roguljа-Pepeonik Z, Fuller JH: Effect of lisinopril on progression of retinopathy in normotensive people with type 1 diabetes. *The Lancet* 351:28-31, 1998
165. Chaturvedi N, Porta M, Klein R, Orchard T, Fuller J, Parving HH, Bilous R, Sjølie AK: Effect of candesartan on prevention (DIRECT-Prevent 1) and progression (DIRECT-Protect 1) of retinopathy in type 1 diabetes: randomised, placebo-controlled trials. *The Lancet* 372:1394-1402, 2008
166. Sjølie AK, Klein R, Porta M, Orchard T, Fuller J, Parving HH, Bilous R, Chaturvedi N: Effect of candesartan on progression and regression of retinopathy in type 2 diabetes (DIRECT-Protect 2): a randomised placebo-controlled trial. *The Lancet* 372:1385-1393, 2008
167. ACCORD Study Group, ACCORD Eye Study Group, Chew EY, Ambrosius WT, Davis MD, Danis RP, Gangaputra S, Greven CM, Hubbard L, Esser BA, Lovato JF, Perdue LH, Goff DC,Jr, Cushman WC, Ginsberg HN, Elam MB, Genuth S, Gerstein HC, Schubart U, Fine LJ: Effects of medical therapies on retinopathy progression in type 2 diabetes. *N Engl J Med* 363:233-244, 2010
168. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL: The Wisconsin Epidemiologic Study of Diabetic Retinopathy: II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol* 102:520-526, 1984

169. Vérier-Mine O, Chaturvedi N, Webb D, Fuller J: Is pregnancy a risk factor for microvascular complications? The EURODIAB Prospective Complications Study. *Diabetic Med* 22:1503-1509, 2005
170. Diabetes Control and Complications Trial Research Group: Effect of pregnancy on microvascular complications in the Diabetes Control and Complications Trial. *Diabetes Care* 23:1084-1091, 2000
171. Chaturvedi N, Stephenson JM, Fuller JH: The relationship between smoking and microvascular complications in the EURODIAB IDDM Complications Study. *Diabetes Care* 18:785-792, 1995
172. Moss SE, Klein R, Klein BE: Cigarette smoking and ten-year progression of diabetic retinopathy. *Ophthalmology* 103:1438-1442, 1996
173. Qiao Q, Keinänen-Kiukaanniemi S, Läärä E: The relationship between hemoglobin levels and diabetic retinopathy. *J Clin Epidemiol* 50:153-158, 1997
174. Porta M, Sjoelie A, Chaturvedi N, Stevens L, Rottiers R, Veglio M, Fuller JH: Risk factors for progression to proliferative diabetic retinopathy in the EURODIAB Prospective Complications Study. *Diabetologia* 44:2203-2209, 2001
175. Krepler K, Biowski R, Schrey S, Jandrasits K, Wedrich A: Cataract surgery in patients with diabetic retinopathy: visual outcome, progression of diabetic retinopathy, and incidence of diabetic macular oedema. *Graefe's archive for clinical and experimental ophthalmology* 240:735-738, 2002
176. Klein BE, Moss SE, Klein R: Is menarche associated with diabetic retinopathy? *Diabetes Care* 13:1034-1038, 1990
177. Young RJ, McCulloch DK, Prescott RJ, Clarke BF: Alcohol: another risk factor for diabetic retinopathy? *BMJ* 288:1035-1037, 1984
178. Lim LS, Wong TY: Lipids and diabetic retinopathy. *Expert Opinion on Biological Therapy* 12:93-105, 2012
179. Klein R, Klein BE, Moss SE: Epidemiology of proliferative diabetic retinopathy. *Diabetes Care* 15:1875-1891, 1992
180. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D, American Diabetes Association: Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care* 28:956-962, 2005
181. Tesfaye S, Stevens L, Stephenson J, Fuller J, Plater M, Ionescu-Tirgoviste C, Nuber A, Pozza G, Ward J: Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study. *Diabetologia* 39:1377-1384, 1996
182. Greene DA, Sima AA, Pfeifer MA, Albers JW: Diabetic neuropathy. *Annu Rev Med* 41:303-317, 1990
183. Quattrini C, Tesfaye S: Understanding the impact of painful diabetic neuropathy. *Diabetes Metab Res* 19:S2-S8, 2003
184. Bril V, England J, Franklin GM, Backonja M, Cohen J, Del Toro D, Feldman E, Iverson D, Perkins B, Russell JW, Zochodne D: Evidence-based guideline: Treatment of painful diabetic neuropathy: report of the American Academy of Neurology, the American Association of Neuromuscular and Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Neurology* 76:1758-1765, 2011
185. Albers JW, Herman WH, Pop-Busui R, Feldman EL, Martin CL, Cleary PA, Waberski BH, Lachin JM, Diabetes Control and Complications Trial /Epidemiology of Diabetes Interventions and Complications Research Group: Effect of prior intensive insulin treatment during the Diabetes Control and Complications Trial (DCCT) on peripheral neuropathy in type 1 diabetes during the Epidemiology of Diabetes Interventions and Complications (EDIC) Study. *Diabetes Care* 33:1090-1096, 2010
186. Gordois A, Scuffham P, Shearer A, Oglesby A, Tobian JA: The health care costs of diabetic peripheral neuropathy in the US. *Diabetes Care* 26:1790-1795, 2003

187. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempler P, Hilsted J, Tesfaye S: Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res* 27:639-653, 2011
188. Gaede P, Vedel P, Larsen N, Jensen GVH, Parving HH, Pedersen O: Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 348:383, 2003
189. Kannel WB, McGee DL: Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care* 2:120-126, 1979
190. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434-444, 1993
191. Snell-Bergeon JK, Hokanson JE, Jensen L, MacKenzie T, Kinney G, Dabelea D, Eckel RH, Ehrlich J, Garg S, Rewers M: Progression of coronary artery calcification in type 1 diabetes: the importance of glycemic control. *Diabetes Care* 26:2923-2928, 2003
192. Cleary PA, Orchard TJ, Genuth S, Wong ND, Detrano R, Backlund JY, Zinman B, Jacobson A, Sun W, Lachin JM, Nathan DM, DCCT/EDIC Research Group: The effect of intensive glycemic treatment on coronary artery calcification in type 1 diabetic participants of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. *Diabetes* 55:3556-3565, 2006
193. Juutilainen A, Lehto S, Rönnemaa T, Pyörälä K, Laakso M: Similarity of the impact of type 1 and type 2 diabetes on cardiovascular mortality in middle-aged subjects. *Diabetes Care* 31:714-719, 2008
194. Krantz JS, Mack WJ, Hodis HN, Liu C, Liu C, Kaufman FR: Early onset of subclinical atherosclerosis in young persons with type 1 diabetes. *J Pediatr* 145:452-457, 2004
195. Larsen J, Brekke M, Bergengen L, Sandvik L, Arnesen H, Hanssen K, Dahl-Jorgensen K: Mean HbA1c over 18 years predicts carotid intima media thickness in women with type 1 diabetes. *Diabetologia* 48:776-779, 2005
196. Krolewski AS, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradley RF, Kahn CR: Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. *Am J Cardiol* 59:750-755, 1987
197. Laing S, Swerdlow A, Slater S, Burden A, Morris A, Waugh N, Gatling W, Bingley P, Patterson C: Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* 46:760-765, 2003
198. Koistinen MJ: Prevalence of asymptomatic myocardial ischaemia in diabetic subjects. *BMJ* 301:92-95, 1990
199. Valsania P, Zarich SW, Kowalchuk GJ, Kosinski E, Warram JH, Krolewski AS: Severity of coronary artery disease in young patients with insulin-dependent diabetes mellitus. *Am Heart J* 122:695-700, 1991
200. Miettinen H, Lehto S, Salomaa V, Mähönen M, Niemelä M, Haffner SM, Pyörälä K, Tuomilehto J: Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA Myocardial Infarction Register Study Group. *Diabetes Care* 21:69-75, 1998
201. Chun BY, Dobson AJ, Heller RF: The impact of diabetes on survival among patients with first myocardial infarction. *Diabetes Care* 20:704-708, 1997
202. Gotto AM,Jr, Pownall HJ, Havel RJ: Introduction to the plasma lipoproteins. *Methods Enzymol* 128:3-41, 1986
203. Dayspring TD: Apoproteins and cell surface receptors regulating lipoprotein metabolism in the setting of type 2 diabetes. In *Lipoproteins in Diabetes Mellitus.* Springer, 2014, p.55-99

204. Sniderman AD, Pedersen T, Kjekshus J: Putting low-density lipoproteins at center stage in atherogenesis. *Am J Cardiol* 79:64-67, 1997
205. Packard CJ, Demant T, Stewart JP, Bedford D, Caslake MJ, Schwertfeger G, Bedynek A, Shepherd J, Seidel D: Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J Lipid Res* 41:305-318, 2000
206. Durrington PN, Bolton CH, Hartog M: Serum and lipoprotein apolipoprotein B levels in normal subjects and patients with hyperlipoproteinaemia. *Clin Chim Acta* 82:151-160, 1978
207. Utermann G: The mysteries of lipoprotein(a). *Science* 246:904-910, 1989
208. Paultre F, Pearson TA, Weil HF, Tuck CH, Myerson M, Rubin J, Francis CK, Marx HF, Philbin EF, Reed RG, Berglund L: High levels of Lp(a) with a small apo(a) isoform are associated with coronary artery disease in African American and white men. *Arterioscler Thromb Vasc Biol* 20:2619-2624, 2000
209. Lewis GF, Rader DJ: New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ Res* 96:1221-1232, 2005
210. Lagrost L, Desrumaux C, Masson D, Deckert V, Gambert P: Structure and function of the plasma phospholipid transfer protein. *Curr Opin Lipidol* 9:203-209, 1998
211. Jian B, de la Llera-Moya M, Ji Y, Wang N, Phillips MC, Swaney JB, Tall AR, Rothblat GH: Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors. *J Biol Chem* 273:5599-5606, 1998
212. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Boren J, Catapano AL, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA, Lesnik P, Masana L, Nordestgaard BG, Ray KK, Reiner Z, Taskinen MR, Tokgozoglu L, Tybjaerg-Hansen A, Watts GF, European Atherosclerosis Society Consensus Panel: Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J* 32:1345-1361, 2011
213. Malmström R, Packard CJ, Caslake M, Bedford D, Stewart P, Yki-Järvinen H, Shepherd J, Taskinen MR: Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes* 47:779-787, 1998
214. Fried SK, Russell CD, Grauso NL, Brolin RE: Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *J Clin Invest* 92:2191-2198, 1993
215. Haas ME, Attie AD, Biddinger SB: The regulation of ApoB metabolism by insulin. *Trends in Endocrinology & Metabolism* 24:391-397, 2013
216. Chait A, Bierman EL, Albers JJ: Low-density lipoprotein receptor activity in cultured human skin fibroblasts. Mechanism of insulin-induced stimulation. *J Clin Invest* 64:1309-1319, 1979
217. Choi SH, Ginsberg HN: Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. *Trends in Endocrinology & Metabolism* 22:353-363, 2011
218. Taskinen MR: Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. *Diabetes* 41:12-17, 1992
219. Dullaart R: Plasma lipoprotein abnormalities in type 1 (insulin-dependent) diabetes mellitus. *Neth J Med* 46:44-54, 1995
220. Weidman SW, Ragland JB, Fisher JN,Jr, Kitabchi AE, Sabesin SM: Effects of insulin on plasma lipoproteins in diabetic ketoacidosis: evidence for a change in high density lipoprotein composition during treatment. *J Lipid Res* 23:171-182, 1982
221. Nikkilä EA, Hormila P: Serum lipids and lipoproteins in insulin-treated diabetes. Demonstration of increased high density lipoprotein concentrations. *Diabetes* 27:1078-1086, 1978

222. Dashti N, Wolfbauer G: Secretion of lipids, apolipoproteins, and lipoproteins by human hepatoma cell line, HepG2: effects of oleic acid and insulin. *J Lipid Res* 28:423-436, 1987
223. Nikkilä EA, Huttunen JK, Ehnholm C: Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. Relationship to plasma triglyceride metabolism. *Diabetes* 26:11-21, 1977
224. Winocour PH, Durrington PN, Ishola M, Anderson DC: Lipoprotein abnormalities in insulin-dependent diabetes mellitus. *Lancet* 1:1176-1178, 1986
225. Kahri J, Groop PH, Viberti G, Elliott T, Taskinen MR: Regulation of Apolipoprotein AI–Containing Lipoproteins in IDDM. *Diabetes* 42:1281-1288, 1993
226. Selam J, Kashyap M, Alberti K, Lozano J, Hanna M, Turner D, Jeandidier N, Chan E, Charles MA: Comparison of intraperitoneal and subcutaneous insulin administration on lipids, apolipoproteins, fuel metabolites, and hormones in type I diabetes mellitus. *Metab Clin Exp* 38:908-912, 1989
227. Duvillard L, Florentin E, Baillot-Rudoni S, Lalanne-Mistrich M, Brun-Pacaud A, Petit J, Brun J, Gambert P, Vergès B: Comparison of apolipoprotein B100 metabolism between continuous subcutaneous and intraperitoneal insulin therapy in type 1 diabetes. *Journal of Clinical Endocrinology & Metabolism* 90:5761-5764, 2005
228. Ruotolo G, Parlavecchia M, Taskinen MR, Galimberti G, Zoppo A, Le NA, Ragogna F, Micossi P, Pozza G: Normalization of lipoprotein composition by intraperitoneal insulin in IDDM. Role of increased hepatic lipase activity. *Diabetes Care* 17:6-12, 1994
229. Bagdade JD, Dunn FL: Improved lipoprotein surface and core lipid composition following intraperitoneal insulin delivery in insulin-dependent diabetes mellitus. *Diabetes Metab* 22:420-426, 1996
230. Thorn LM, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, Saraheimo M, Wadén J, Rönnback M, Rosengård-Bärlund M, Björkesten CG: Metabolic syndrome in type 1 diabetes. *Diabetes Care* 28:2019-2024, 2005
231. Becker B, Kronenberg F, Kielstein JT, Haller H, Morath C, Ritz E, Fliser D, MMKD Study Group: Renal insulin resistance syndrome, adiponectin and cardiovascular events in patients with kidney disease: the mild and moderate kidney disease study. *J Am Soc Nephrol* 16:1091-1098, 2005
232. Kilpatrick ES, Rigby AS, Atkin SL: Insulin resistance, the metabolic syndrome, and complication risk in type 1 diabetes "Double diabetes" in the Diabetes Control and Complications Trial. *Diabetes Care* 30:707-712, 2007
233. Groop PH, Forsblom C, Thomas MC: Mechanisms of disease: pathway-selective insulin resistance and microvascular complications of diabetes. *Nature Reviews Endocrinology* 1:100-110, 2005
234. Taskinen MR: Lipoprotein lipase in diabetes. *Diabetes Metab Rev* 3:551-570, 1987
235. Syvänne M, Taskinen MR: Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. *The Lancet* 350:S20-S23, 1997
236. Tan KC, Cooper MB, Ling K, Griffin BA, Freeman DJ, Packard CJ, Shepherd J, Hales CN, Betteridge DJ: Fasting and postprandial determinants for the occurrence of small dense LDL species in non-insulin-dependent diabetic patients with and without hypertriglyceridaemia: the involvement of insulin, insulin precursor species and insulin resistance. *Atherosclerosis* 113:273-287, 1995
237. Haffner S, Mykkänen L, Robbins D, Valdez R, Miettinen H, Howard B, Stern M, Bowsher R: A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non-diabetic subjects. *Diabetologia* 38:1328-1336, 1995
238. Alabakovska S, Labudovic D, Tosheska K, Spiroski M, Todorova B: Low density lipoprotein subclass distribution in children with diabetes mellitus. *Bratislavské lekárske listy* 109:155-159, 2008

239. Berneis KK, Krauss RM: Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res* 43:1363-1379, 2002
240. Anber V, Griffin B, McConnell M, Packard C, Shepherd J: Influence of plasma lipid and LDL-subfraction profile on the interaction between low density lipoprotein with human arterial wall proteoglycans. *Atherosclerosis* 124:261-271, 1996
241. Tani M, Kawakami A, Mizuno Y, Imase R, Ito Y, Kondo K, Ishii H, Yoshida M: Small dense LDL enhances THP-1 macrophage foam cell formation. *J Atheroscler Thromb* 18:698-704, 2011
242. Younis N, Charlton-Menys V, Sharma R, Soran H, Durrington PN: Glycation of LDL in non-diabetic people: Small dense LDL is preferentially glycated both in vivo and in vitro. *Atherosclerosis* 202:162-168, 2009
243. de Graaf J, Hak-Lemmers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF: Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler Thromb* 11:298-306, 1991
244. Tribble DL, Holl LG, Wood PD, Krauss RM: Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 93:189-199, 1992
245. Palinski W, Tsimikas S: Immunomodulatory effects of statins: mechanisms and potential impact on arteriosclerosis. *J Am Soc Nephrol* 13:1673-1681, 2002
246. Sniderman AD, Scantlebury T, Cianflone K: Hypertriglyceridemic hyperapoB: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. *Ann Intern Med* 135:447-459, 2001
247. Hopkins GJ, Barter PJ: Role of triglyceride-rich lipoproteins and hepatic lipase in determining the particle size and composition of high density lipoproteins. *J Lipid Res* 27:1265-1277, 1986
248. Singh K, Raghavan VA: Insulin resistance and atherosclerosis. In *Lipoproteins in Diabetes Mellitus*. Springer, 2014, p.41-54
249. Drew BG, Rye K, Duffy SJ, Barter P, Kingwell BA: The emerging role of HDL in glucose metabolism. *Nature Reviews Endocrinology* 8:237-245, 2012
250. Eckel RH, Albers JJ, Cheung MC, Wahl PW, Lindgren FT, Bierman EL: High density lipoprotein composition in insulin-dependent diabetes mellitus. *Diabetes* 30:132-138, 1981
251. Adiels M, Olofsson SO, Taskinen MR, Boren J: Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 28:1225-1236, 2008
252. Hashemi M, Saadat M, Behjati M, Kelishadi R: Comparison of serum Apolipoprotein levels of diabetic children and healthy children with or without diabetic parents. *Cholesterol* 2012: Article ID 490381:doi:10.1155/2012/490381, 2012
253. Cobble M, Mize PD, Brinton EA: Lipoprotein subclasses and cardiovascular disease risk in insulin-resistant diabetes. In *Lipoproteins in Diabetes Mellitus*. Springer, 2014, p.11-40
254. Caron S, Verrijken A, Mertens I, Samanez CH, Mautino G, Haas JT, Duran-Sandoval D, Prawitt J, Francque S, Vallez E, Muhr-Tailleux A, Berard I, Kuipers F, Kuivenhoven JA, Biddinger SB, Taskinen MR, Van Gaal L, Staels B: Transcriptional activation of apolipoprotein CIII expression by glucose may contribute to diabetic dyslipidemia. *Arterioscler Thromb Vasc Biol* 31:513-519, 2011
255. Curtiss LK, Witztum JL: Plasma apolipoproteins AI, AII, B, CI, and E are glucosylated in hyperglycemic diabetic subjects. *Diabetes* 34:452-461, 1985
256. Orth SR, Ritz E: The nephrotic syndrome. *N Engl J Med* 338:1202-1211, 1998
257. Skrzep-Poloczek B, Tomasik A, Tarnawski R, Hyla-Klekot L, Dyduch A, Wojciechowska C, Wesolowski W, Kopieczna-Grzebieniak E, Zalejska-Fiolka J, Widera E: Nephrotic origin hyperlipidemia, relative reduction of vitamin E level and subsequent oxidative stress may promote atherosclerosis. *Nephron* 89:68-72, 2001

258. Davies RW, Staprans I, Hutchison FN, Kaysen GA: Proteinuria, not altered albumin metabolism, affects hyperlipidemia in the nephrotic rat. *J Clin Invest* 86:600-605, 1990
259. Wanner C, Rader D, Bartens W, Kramer J, Brewer HB, Schollmeyer P, Wieland H: Elevated plasma lipoprotein (a) in patients with the nephrotic syndrome. *Ann Intern Med* 119:263-269, 1993
260. Gansevoort RT, Heeg JE, Dikkeschei FD, de Zeeuw D, de Jong PE, Dullaart RP: Symptomatic antiproteinuric treatment decreases serum lipoprotein (a) concentration in patients with glomerular proteinuria. *Nephrol Dial Transplant* 9:244-250, 1994
261. Vaziri ND, Liang K, Parks JS: Acquired lecithin-cholesterol acyltransferase deficiency in nephrotic syndrome. *Am J Physiol Renal Physiol* 280:F823-F828, 2001
262. Diamond JR, Karnovsky MJ: Focal and segmental glomerulosclerosis: Analogies to atherosclerosis. *Kidney Int* 33:917-924, 1988
263. Myhre E, Gjone E, Flatmark A, Hovig T: Renal failure in familial lecithin-cholesterol acyltransferase deficiency. *Nephron* 18:239-248, 1977
264. Oikawa S, Matsunaga A, Saito T, Sato H, Seki T, Hoshi K, Hayasaka K, Kotake H, Midorikawa H, Sekikawa A, Hara S, Abe K, Toyota T, Jingami H, Nakamura H, Sasaki J: Apolipoprotein E Sendai (arginine 145->proline): a new variant associated with lipoprotein glomerulopathy. *J Am Soc Nephrol* 8:820-823, 1997
265. Suzaki K, Kobori S, Ueno S, Uehara M, Kayashima T, Takeda H, Fukuda S, Takahashi K, Nakamura N, Uzawa H: Effects of plasmapheresis on familial type III hyperlipoproteinemia associated with glomerular lipidosis, nephrotic syndrome and diabetes mellitus. *Atherosclerosis* 80:181-189, 1990
266. Peric-Golia L, Peric-Golia M: Aortic and renal lesions in hypercholesterolemic adult, male, virgin Sprague-Dawley rats. *Atherosclerosis* 46:57-65, 1983
267. Keane WF, Kasiske BL, O'Donnell MP, Kim Y: The role of altered lipid metabolism in the progression of renal disease: experimental evidence. *Am J Kidney Dis* 17:38-42, 1991
268. Rovin BH, Tan LC: LDL stimulates mesangial fibronectin production and chemoattractant expression. *Kidney Int* 43:218-225, 1993
269. Nishida Y, Oda H, Yorioka N: Effect of lipoproteins on mesangial cell proliferation. *Kidney Int* 56:S51-S53, 1999
270. Lynn EG, Siow YL: Very low-density lipoprotein stimulates the expression of monocyte chemoattractant protein-1 in mesangial cells. *Kidney Int* 57:1472-1483, 2000
271. Furuta T, Saito T, Ootaka T, Soma J, Obara K, Abe K, Yoshinaga K: The role of macrophages in diabetic glomerulosclerosis. *Am J Kidney Dis* 21:480-485, 1993
272. Giacco F, Brownlee M: Oxidative stress and diabetic complications. *Circ Res* 107:1058-1070, 2010
273. Veiraiah A: Hyperglycemia, lipoprotein glycation, and vascular disease. *Angiology* 56:431-438, 2005
274. Rutledge JC, Ng KF, Aung HH, Wilson DW: Role of triglyceride-rich lipoproteins in diabetic nephropathy. *Nature Reviews Nephrology* 6:361-370, 2010
275. Vasconcelos EMA, Degasperi GR, de Oliveira HCF, Vercesi AE, de Faria EC, Castilho LN: Reactive oxygen species generation in peripheral blood monocytes and oxidized LDL are increased in hyperlipidemic patients. *Clin Biochem* 42:1222-1227, 2009
276. Keane WF, O'donnell MP, Kasiske BL, Kim Y: Oxidative modification of low-density lipoproteins by mesangial cells. *J Am Soc Nephrol* 4:187-194, 1993
277. Ruan XZ, Varghese Z, Moorhead JF: An update on the lipid nephrotoxicity hypothesis. *Nature Reviews Nephrology* 5:713-721, 2009
278. Sharma P, Reddy K, Franki N, Sanwal V, Sankaran R, Ahuja TS, Gibbons N, Mattana J, Singhal PC: Native and oxidized low density lipoproteins modulate mesangial cell apoptosis. *Kidney Int* 50:1604-1611, 1996

279. Dimmeler S, Haendeler J, Galle J, Zeiher AM: Oxidized low-density lipoprotein induces apoptosis of human endothelial cells by activation of CPP32-like proteases: a mechanistic clue to the 'response to injury' hypothesis. *Circulation* 95:1760-1763, 1997
280. Bussolati B, Deregibus MC, Fonsato V, Doublier S, Spatola T, Procida S, Di Carlo F, Camussi G: Statins prevent oxidized LDL-induced injury of glomerular podocytes by activating the phosphatidylinositol 3-kinase/AKT-signaling pathway. *J Am Soc Nephrol* 16:1936-1947, 2005
281. Keane WF: The role of lipids in renal disease: future challenges. *Kidney Int* 57:S27-S31, 2000
282. Jeansson M, Haraldsson B: Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. *Am J Physiol Renal Physiol* 290:F111-F116, 2006
283. Ding G, Van Goor H, Ricardo SD, Orlowski JM, Diamond JR: Oxidized LDL stimulates the expression of TGF-β and fibronectin in human glomerular epithelial cells. *Kidney Int* 51:147-154, 1997
284. Choi ME: Mechanism of transforming growth factor-beta1 signaling: Role of the mitogen-activated protein kinase. *Kidney Int* 58:S53-S58, 2000
285. Bondi CD, Manickam N, Lee DY, Block K, Gorin Y, Abboud HE, Barnes JL: NAD(P)H oxidase mediates TGF-beta1-induced activation of kidney myofibroblasts. *J Am Soc Nephrol* 21:93-102, 2010
286. Misra A, Kumar S, Kishore Vikram N, Kumar A: The role of lipids in the development of diabetic microvascular complications: implications for therapy. *Am J Cardiovasc Drugs* 3:325-338, 2003
287. Lennernäs H, Fager G: Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. *Clin Pharmacokinet* 32:403-425, 1997
288. Collins R, Armitage J, Parish S, Sleigh P, Peto R, Heart Protection Study Collaborative G: MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet* 361:2005-2016, 2003
289. Sever PS, Poulter NR, Dahlof B, Wedel H, Collins R, Beevers G, Caulfield M, Kjeldsen SE, Kristinsson A, McInnes GT, Mehlsen J, Nieminen M, O'Brien E, Ostergren J: Reduction in cardiovascular events with atorvastatin in 2,532 patients with type 2 diabetes: Anglo-Scandinavian Cardiac Outcomes Trial - lipid-lowering arm (ASCOT-LLA). *Diabetes Care* 28:1151-1157, 2005
290. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, W Neil HA, Livingstone SJ, Thomason MJ, Mackness MI, Charlton-Menys V, Fuller JH: Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *The Lancet* 364:685-696, 2004
291. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial: Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). *JAMA* 288:2998-3007, 2002
292. Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, Armitage J, Baigent C: Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. *Lancet* 371:117-125, 2008
293. Mihaylova B, Briggs A, Armitage J, Parish S, Gray A, Collins R: Heart Protection Study Collaborative. Lifetime cost effectiveness of simvastatin in a range of risk groups and age groups derived from a randomised trial of 20,536 people. *BMJ* 333:1145-1149, 2006

294. Auwerx J, Schoonjans K, Fruchart JC, Staels B: Regulation of triglyceride metabolism by PPARs: fibrates and thiazolidinediones have distinct effects. *J Atheroscler Thromb* 3:81-89, 1996
295. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesäniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M, FIELD Study Investigators: Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD Study): randomised controlled trial. *Lancet* 366:1849-1861, 2005
296. Scott R, O'Brien R, Fulcher G, Pardy C, D'Emden M, Tse D, Taskinen MR, Ehnholm C, Keech A, Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study Investigators: Effects of fenofibrate treatment on cardiovascular disease risk in 9,795 individuals with type 2 diabetes and various components of the metabolic syndrome: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study. *Diabetes Care* 32:493-498, 2009
297. Ginsberg H, Elam M, Lovato L, Crouse 3rd J, Leiter L, Linz P, Friedewald W, Buse J, Gerstein H, Probstfield J: Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med* 362:1563-1574, 2010
298. Tonelli M, Isles C, Craven T, Tonkin A, Pfeffer MA, Shepherd J, Sacks FM, Furberg C, Cobbe SM, Simes J, West M, Packard C, Curhan GC: Effect of pravastatin on rate of kidney function loss in people with or at risk for coronary disease. *Circulation* 112:171-178, 2005
299. Huskey J, Lindenfeld J, Cook T, Targher G, Kendrick J, Kjekshus J, Pedersen T, Chonchol M: Effect of simvastatin on kidney function loss in patients with coronary heart disease: findings from the Scandinavian Simvastatin Survival Study (4S). *Atherosclerosis* 205:202-206, 2009
300. Shepherd J, Kastelein JJ, Bittner V, Deedwania P, Breazna A, Dobson S, Wilson DJ, Zuckerman A, Wenger NK, Treating to New Targets I: Effect of intensive lipid lowering with atorvastatin on renal function in patients with coronary heart disease: the Treating to New Targets (TNT) Study. *Clin J Am Soc Nephrol* 2:1131-1139, 2007
301. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HAW, Livingstone SJ, Charlton-Menys V, DeMicco DA, Fuller JH: Effects of atorvastatin on kidney outcomes and cardiovascular disease in patients with diabetes: an analysis from the Collaborative Atorvastatin Diabetes Study (CARDS). *American Journal of Kidney Diseases* 54:810-819, 2009
302. Shepherd J, Kastelein JJ, Bittner VA, Carmena R, Deedwania PC, Breazna A, Dobson S, Wilson DJ, Zuckerman AL, Wenger NK: Intensive lipid lowering with atorvastatin in patients with coronary artery disease, diabetes, and chronic kidney disease. *Mayo Clin Proc* 83:870-879, 2008
303. Sandhu S, Wiebe N, Fried LF, Tonelli M: Statins for improving renal outcomes: a meta-analysis. *J Am Soc Nephrol* 17:2006-2016, 2006
304. Geng Q, Ren J, Song J, Li S, Chen H: Meta-analysis of the Effect of Statins on Renal Function. *Am J Cardiol* 114:562-570, 2014
305. Ridker PM, MacFadyen J, Cressman M, Glynn RJ: Efficacy of rosuvastatin among men and women with moderate chronic kidney disease and elevated high-sensitivity C-reactive protein: a secondary analysis from the JUPITER (Justification for the Use of Statins in Prevention–an Intervention Trial Evaluating Rosuvastatin) Trial. *J Am Coll Cardiol* 55:1266-1273, 2010
306. Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, Wanner C, Krane V, Cass A, Craig J: The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *The Lancet* 377:2181-2192, 2011

307. Holdaas H, Fellström B, Jardine AG, Holme I, Nyberg G, Fauchald P, Grönhagen-Riska C, Madsen S, Neumayer HH, Cole E: Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. *The Lancet* 361:2024-2031, 2003
308. Fellström B, Holdaas H, Jardine AG, Holme I, Nyberg G, Fauchald P, Grönhagen-Riska C, Madsen S, Neumayer HH, Cole E: Effect of fluvastatin on renal end points in the Assessment of Lescol in Renal Transplant (ALERT) trial. *Kidney Int* 66:1549-1555, 2004
309. Takagi H, Umemoto T: A meta-analysis of randomized trials for effects of atorvastatin on renal function in chronic kidney disease. *Int J Cardiol* 152:242-244, 2011
310. Davis TME, Ting R, Best J, Donoghoe M, Drury P, Sullivan D, Jenkins A, O'Connell R, Whiting M, Glasziou P: Effects of fenofibrate on renal function in patients with type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study. *Diabetologia* 54:280-290, 2011
311. Mychaleckyj JC, Craven T, Nayak U, Buse J, Crouse JR, Elam M, Kirchner K, Lorber D, Marcovina S, Sivitz W, Sperl-Hillen J, Bonds DE, Ginsberg HN: Reversibility of fenofibrate therapy-induced renal function impairment in ACCORD type 2 diabetic participants. *Diabetes Care* 35:1008-1014, 2012
312. Hottelart C, el Esper N, Achard JM, Pruna A, Fournier A: Fenofibrate increases blood creatinine, but does not change the glomerular filtration rate in patients with mild renal insufficiency. *Nephrologie* 20:41-44, 1999
313. Forsblom C, Hiukka A, Leinonen ES, Sundvall J, Groop PH, Taskinen MR: Effects of Long-Term Fenofibrate Treatment on Markers of Renal Function in Type 2 Diabetes: FIELD Helsinki Substudy. *Diabetes Care* 33:215-220, 2009
314. Ansquer JC, Foucher C, Rattier S, Taskinen MR, Steiner G, DAIS Investigators: Fenofibrate reduces progression to microalbuminuria over 3 years in a placebo-controlled study in type 2 diabetes: results from the Diabetes Atherosclerosis Intervention Study (DAIS). *Am J Kidney Dis* 45:485-493, 2005
315. Jun M, Zhu B, Tonelli M, Jardine MJ, Patel A, Neal B, Liyanage T, Keech A, Cass A, Perkovic V: Effects of fibrates in kidney disease: a systematic review and meta-analysis. *J Am Coll Cardiol* 60:2061-2071, 2012
316. Cohen JC, Boerwinkle E, Mosley TH,Jr, Hobbs HH: Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 354:1264-1272, 2006
317. Law MR, Wald NJ, Rudnicka AR: Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ* 326:1423, doi: http://dx.doi.org/10.1136/bmj.326.7404.1423, 2003
318. Zoja C, Corna D, Rottoli D, Cattaneo D, Zanchi C, Tomasoni S, Abbate M, Remuzzi G: Effect of combining ACE inhibitor and statin in severe experimental nephropathy. *Kidney Int* 61:1635-1645, 2002
319. Adolescent type 1 Diabetes cardio-renal Intervention Trial Research Group: Adolescent type 1 Diabetes Cardio-renal Intervention Trial (AdDIT). *BMC Pediatr* 9:79, doi:10.1186/1471-2431-9-79, 2009
320. Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, Seshasai SRK, McMurray JJ, Freeman DJ, Jukema JW: Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *The Lancet* 375:735-742, 2010
321. Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A: Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 103:357-362, 2001
322. Ridker PM, Pradhan A, MacFadyen JG, Libby P, Glynn RJ: Cardiovascular benefits and diabetes risks of statin therapy in primary prevention: an analysis from the JUPITER trial. *The Lancet* 380:565-571, 2012

323. Strandberg TE, Pienimäki T, Strandberg AY, Pitkälä KH, Tilvis RS: Association Between Use of Statin Medication and Weight Change in Older Men. *J Am Geriatr Soc* 60:1588-1590, 2012
324. Swerdlow DI, Preiss D, Kuchenbaecker KB, Holmes MV, Engmann JE, Shah T, Sofat R, Stender S, Johnson PC, Scott RA: HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *The Lancet* doi: 10.1016/S0140-6736(14)61183-1, 2014
325. Fagerudd J, Forsblom C, Pettersson-Fernholm K, Groop PH: Implementation of guidelines for the prevention of diabetic nephropathy. *Diabetes Care* 27:803-804, 2004
326. Hutchinson A, McIntosh A, Peters J, O'keeffe C, Khunti K, Baker R, Booth A: Effectiveness of screening and monitoring tests for diabetic retinopathy–a systematic review. *Diabetic Med* 17:495-506, 2000
327. Davis MD, Fisher MR, Gangnon RE, Barton F, Aiello LM, Chew EY, Ferris 3rd FL, Knatterud GL: Risk factors for high-risk proliferative diabetic retinopathy and severe visual loss: Early Treatment Diabetic Retinopathy Study Report# 18. *Invest Ophthalmol Vis Sci* 39:233-252, 1998
328. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Katus HA, Apple FS, Lindahl B, Morrow DA: Third universal definition of myocardial infarction. *J Am Coll Cardiol* 60:1581-1598, 2012
329. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499-502, 1972
330. Fine JP, Gray RJ: A proportional hazards model for the subdistribution of a competing risk. *Journal of the American Statistical Association* 94:496-509, 1999
331. Sundström J, Byberg L, Gedeborg R, Michaëlsson K, Berglund L: Useful tests of usefulness of new risk factors: tools for assessing reclassification and discrimination. *Scand J Public Health* 39:439-441, 2011
332. Greenland P, Smith Jr SC, Grundy SM: Improving Coronary Heart Disease Risk Assessment in Asymptomatic People Role of Traditional Risk Factors and Noninvasive Cardiovascular Tests. *Circulation* 104:1863-1867, 2001
333. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation,and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III): Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 106:3143-3421, 2002
334. Inadequate NA: JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* 91:v1-v52, 2005
335. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, Witztum JL: Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. *J Am Coll Cardiol* 51:1512-1524, 2008
336. Nikkilä EA, Kekki M: Plasma triglyceride transport kinetics in diabetes mellitus. *Metabolism* 22:1-22, 1973
337. Lipid and lipoprotein levels in patients with IDDM diabetes control and complication. Trial experience. The DCCT Research Group. *Diabetes Care* 15:886-894, 1992
338. Doggen K, Nobels F, Scheen AJ, Van Crombrugge P, Van Casteren V, Mathieu C: Cardiovascular risk factors and complications associated with albuminuria and impaired renal function in insulin-treated diabetes. *J Diabetes Complications* 27:370-375, 2013
339. Yamashita T, Makino H, Nakatani R, Ohata Y, Miyamoto Y, Kishimoto I: Renal insufficiency without albuminuria is associated with peripheral artery atherosclerosis and lipid metabolism disorders in patients with type 2 diabetes. *J Atheroscler Thromb* 20:790-797, 2013

340. Kim HJ, Jee SH, Lee SJ, Park E, Kim S, Jo JS, Yun JE, Lee GJ: The association of serum lipids with renal function: the Korea Medical Institute Study. *European Journal of Cardiovascular Prevention & Rehabilitation* 16:60-65, 2009
341. Mattock MB, Cronin N, Cavallo-Perin P, Idzior-Walus B, Penno G, Bandinelli S, Standl E, Kofinis A, Fuller JH, EURODIAB IDDM Complications Study: Plasma lipids and urinary albumin excretion rate in type 1 diabetes mellitus: the EURODIAB IDDM Complications Study. *Diabetic Med* 18:59-67, 2001
342. Estudio Diamante: Renal involvement in type 1 (IDDM) diabetes in Spain. *Diabetes Res Clin Pract* 38:129-137, 1997
343. Caramori ML, Fioretto P, Mauer M: Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. *Diabetes* 52:1036-1040, 2003
344. Trofimenko II, Dobronravov VA, Bystrova NN, Drozdova YV, Galkina OV, Smirnov AV: Prevalence of subnormal glomerular filtiation rate in patients with diabetes mellitus. *Ter Arkh* 80:48-52, 2008
345. Thomas MC, MacIsaac RJ, Jerums G, Weekes A, Moran J, Shaw JE, Atkins RC: Nonalbuminuric renal impairment in type 2 diabetic patients and in the general population (National Evaluation of the Frequency of Renal impairment cO-existing with NIDDM [NEFRON] 11). *Diabetes Care* 32:1497-1502, 2009
346. Penno G, Solini A, Bonora E, Fondelli C, Orsi E, Zerbini G, Trevisan R, Vedovato M, Gruden G, Cavalot F: Clinical significance of nonalbuminuric renal impairment in type 2 diabetes. *J Hypertens* 29:1802-1809, 2011
347. Retnakaran R, Cull CA, Thorne KI, Adler AI, Holman RR, UKPDS Study Group: Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. *Diabetes* 55:1832-1839, 2006
348. Yokoyama H, Sone H, Oishi M, Kawai K, Fukumoto Y, Kobayashi M, Japan Diabetes Clinical Data Management: Prevalence of albuminuria and renal insufficiency and associated clinical factors in type 2 diabetes: the Japan Diabetes Clinical Data Management study (JDDM15). *Nephrology Dialysis Transplantation* 24:1212-1219, 2009
349. MacIsaac R, Tsalamandris C, Panagiotopoulos S, Smith T, McNeil K, Jerums G: Nonalbuminuric renal insufficiency in type 2 diabetes. *Diabetes Care* 27:195-200, 2004
350. Pavkov ME, Mason CC, Bennett PH, Curtis JM, Knowler WC, Nelson RG: Change in the distribution of albuminuria according to estimated glomerular filtration rate in Pima Indians with type 2 diabetes. *Diabetes Care* 32:1845-1850, 2009
351. Kramer H, Nguyen Q, Curhan G, Hsu C: Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA* 289:3273-3277, 2003
352. MacIsaac RJ, Panagiotopoulos S, McNeil KJ, Smith TJ, Tsalamandris C, Hao H, Matthews PG, Thomas MC, Power DA, Jerums G: Is nonalbuminuric renal insufficiency in type 2 diabetes related to an increase in intrarenal vascular disease? *Diabetes Care* 29:1560-1566, 2006
353. Fioretto P, Caramori M, Mauer M: The kidney in diabetes: dynamic pathways of injury and repair. The Camillo Golgi Lecture 2007. *Diabetologia* 51:1347-1355, 2008
354. Amin AP, Whaley-Connell AT, Li S, Chen S, McCullough PA, Kosiborod MN, KEEP Investigators: The Synergistic Relationship Between Estimated GFR and Microalbuminuria in Predicting Long-term Progression to ESRD or Death in Patients With Diabetes: Results From the Kidney Early Evaluation Program (KEEP). *American Journal of Kidney Diseases* 61:S12-S23, 2013
355. Chaturvedi N, Bandinelli S, Mangili R, Penno G, Rottiers RE, Fuller JH: Microalbuminuria in type 1 diabetes: rates, risk factors and glycemic threshold. *Kidney Int* 60:219-227, 2001

356. Giorgino F, Laviola L, Cavallo Perin P, Solnica B, Fuller J, Chaturvedi N: Factors associated with progression to macroalbuminuria in microalbuminuric Type 1 diabetic patients: the EURODIAB Prospective Complications Study. *Diabetologia* 47:1020-1028, 2004
357. de Boer IH, Paterson AD, Brunzell JD: Long-term Renal Outcomes of Patients With Type 1 Diabetes Mellitus and Microalbuminuria: An Analysis of the DCCT/EDIC Cohort reply. *Arch Intern Med* 171:1597-1597, 2011
358. Raile K, Galler A, Hofer S, Herbst A, Dunstheimer D, Busch P, Holl RW: Diabetic nephropathy in 27,805 children, adolescents, and adults with type 1 diabetes. *Diabetes Care* 30:2523-2528, 2007
359. Hovind P, Rossing P, Tarnow L, Smidt UM, Parving HH: Progression of diabetic nephropathy. *Kidney Int* 59:702-709, 2001
360. Colhoun HM, Lee ET, Bennett PH, Lu M, Keen H, Wang SL, Stevens LK, Fuller JH: Risk factors for renal failure: the WHO Mulinational Study of Vascular Disease in Diabetes. *Diabetologia* 44:S46-S53, 2001
361. Afghahi H, Cederholm J, Eliasson B, Zethelius B, Gudbjornsdottir S, Hadimeri H, Svensson MK: Risk factors for the development of albuminuria and renal impairment in type 2 diabetes - the Swedish National Diabetes Register (NDR). *Nephrol Dial Transplant* 26:1236-1243, 2011
362. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS: Regression of microalbuminuria in type 1 diabetes. *N Engl J Med* 348:2285-2293, 2003
363. Schaeffner ES, Kurth T, Curhan GC, Glynn RJ, Rexrode KM, Baigent C, Buring JE, Gaziano JM: Cholesterol and the risk of renal dysfunction in apparently healthy men. *J Am Soc Nephrol* 14:2084-2091, 2003
364. Mulec H, Johnsen SA, Wiklund O, Bjorck S: Cholesterol: a renal risk factor in diabetic nephropathy? *Am J Kidney Dis* 22:196-201, 1993
365. Samuelsson O, Mulec H, Knight-Gibson C, Attman P, Kron B, Larsson R, Weiss L, Wedel H, Alaupovic P: Lipoprotein abnormalities are associated with increased rate of progression of human chronic renal insufficiency. *Nephrology Dialysis Transplantation* 12:1908-1915, 1997
366. Mänttäri M, Tiula E, Alikoski T, Manninen V: Effects of hypertension and dyslipidemia on the decline in renal function. *Hypertension* 26:670-675, 1995
367. Lyons TJ, Jenkins AJ, Zheng D, Lackland DT, McGee D, Garvey WT, Klein RL: Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. *Invest Ophthalmol Vis Sci* 45:910, 2004
368. Sinav S, Onelge MA, Onelge S, Sinav B: Plasma lipids and lipoproteins in retinopathy of type I (insulin-dependent) diabetic patients. *Ann Ophthalmol* 25:64-66, 1993
369. Klein BE, Moss SE, Klein R, Surawicz TS: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XIII. Relationship of serum cholesterol to retinopathy and hard exudate. *Ophthalmology* 98:1261-1265, 1991
370. Chaturvedi N, Sjoelie AK, Porta M, Aldington SJ, Fuller JH, Songini M, Kohner EM, EURODIAB Prospective Complications Study: Markers of insulin resistance are strong risk factors for retinopathy incidence in type 1 diabetes. *Diabetes Care* 24:284-289, 2001
371. Lloyd CE, Klein R, Maser RE, Kuller LH, Becker DJ, Orchard TJ: The progression of retinopathy over 2 years: the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study. *J Diabetes Complications* 9:140-148, 1995
372. Cohen RA, Hennekens CH, Christen WG, Krolewski A, Nathan DM, Peterson MJ, LaMotte F, Manson JAE: Determinants of retinopathy progression in type 1 diabetes mellitus. *Am J Med* 107:45-51, 1999
373. Miljanovic B, Glynn RJ, Nathan DM, Manson JAE, Schaumberg DA: A prospective study of serum lipids and risk of diabetic macular edema in type 1 diabetes. *Diabetes* 53:2883-2892, 2004

374. Chew EY, Klein ML, Ferris III FL, Remaley NA, Murphy RP, Chantry K, Hoogwerf BJ, Miller D: Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy: Early Treatment Diabetic Retinopathy Study (ETDRS) Report 22. *Arch Ophthalmol* 114:1079-1084, 1996
375. van Leiden HA, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, Stehouwer CDA, Polak BCP: Blood pressure, lipids, and obesity are associated with retinopathy. *Diabetes Care* 25:1320-1325, 2002
376. Klein R, Sharrett AR, Klein BEK, Moss SE, Folsom AR, Wong TY, Brancati FL, Hubbard LD, Couper D: The association of atherosclerosis, vascular risk factors, and retinopathy in adults with diabetes: the Atherosclerosis Risk in Communities Study. *Ophthalmology* 109:1225-1234, 2002
377. Stephenson JM, Fuller JH, Viberti GC, Sjolie AK, Navalesi R: Blood pressure, retinopathy and urinary albumin excretion in IDDM: the EURODIAB IDDM Complications Study. *Diabetologia* 38:599-603, 1995
378. Group UKPDS: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317:703-713, 1998
379. Soedamah-Muthu SS, Chaturvedi N, Toeller M, Ferriss B, Reboldi P, Michel G, Manes C, Fuller JH: Risk factors for coronary heart disease in type 1 diabetic patients in Europe The EURODIAB Prospective Complications Study. *Diabetes Care* 27:530-537, 2004
380. Orchard TJ, Olson JC, Erbey JR, Williams K, Forrest KYZ, Smithline Kinder L, Ellis D, Becker DJ: Insulin Resistance–Related Factors, but not Glycemia, Predict Coronary Artery Disease in Type 1 Diabetes 10-year follow-up data from the Pittsburgh Epidemiology of Diabetes Complications study. *Diabetes Care* 26:1374-1379, 2003
381. Grauslund J, Jørgensen TM, Nybo M, Green A, Rasmussen LM, Sjølie AK: Risk factors for mortality and ischemic heart disease in patients with long-term type 1 diabetes. *J Diabetes Complications* 24:223-228, 2010
382. Fuller J, Stevens L, Wang S: Risk factors for cardiovascular mortality and morbidity: The WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* 44:S54-S64, 2001
383. Shaikh M, Wootton R, Nordestgaard BG, Baskerville P, Lumley JS, La Ville AE, Quiney J, Lewis B: Quantitative studies of transfer in vivo of low density, Sf 12-60, and Sf 60-400 lipoproteins between plasma and arterial intima in humans. *Arterioscler Thromb* 11:569-577, 1991
384. Nordestgaard BG, Tybjaerg-Hansen A, Lewis B: Influx in vivo of low density, intermediate density, and very low density lipoproteins into aortic intimas of genetically hyperlipidemic rabbits. Roles of plasma concentrations, extent of aortic lesion, and lipoprotein particle size as determinants. *Arterioscler Thromb* 12:6-18, 1992
385. Trialists CT: Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170 000 participants in 26 randomised trials. *The Lancet* 376:1670-1681, 2010
386. Tighe DA, Ockene IS, Reed G, Nicolosi R: Calculated low density lipoprotein cholesterol levels frequently underestimate directly measured low density lipoprotein cholesterol determinations in patients with serum triglyceride levels $\leq$ 4.52 mmol/l: An analysis comparing the LipiDirect® magnetic LDL assay with the Friedewald calculation. *Clinica chimica acta* 365:236-242, 2006
387. Rubies-Prat J, Reverter JL, Senti M, Pedro-Botet J, Salinas I, Lucas A, Nogues X, Sanmarti A: Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus. *Diabetes Care* 16:1081-1086, 1993

388. Martin SS, Blaha MJ, Elshazly MB, Brinton EA, Toth PP, McEvoy JW, Joshi PH, Kulkarni KR, Mize PD, Kwiterovich PO: Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. *J Am Coll Cardiol* 62:732-739, 2013
389. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Hólm H, Ding EL, Johnson T: Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *The Lancet* 380:572-580, 2012
390. Olson JC, Erbey JR, Forrest KY, Williams K, Becker DJ, Orchard TJ: Glycemia (or, in women, estimated glucose disposal rate) predict lower extremity arterial disease events in type 1 diabetes. *Metab Clin Exp* 51:248-254, 2002
391. Stevens LA, Coresh J, Greene T, Levey AS: Assessing kidney function - measured and estimated glomerular filtration rate. *N Engl J Med* 354:2473-2483, 2006
392. Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RG, Rahman M, Deysher AE, Zhang YL, Schmid CH: Evaluation of the modification of diet in renal disease study equation in a large diverse population. *J Am Soc Nephrol* 18:2749-2757, 2007
393. Poggio ED, Wang X, Greene T, Van Lente F, Hall PM: Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. *J Am Soc Nephrol* 16:459-466, 2005
394. Parving HH, Hommel E: Prognosis in diabetic nephropathy. *BMJ* 299:230-233, 1989
395. The Emerging Risk Factors Collaboration: Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 302:1993-2000, 2009
396. Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J: Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet* 45:1345-1352, 2013
397. Varbo A, Benn M, Tybjaerg-Hansen A, Nordestgaard BG: Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation* 128:1298-1309, 2013
398. Jiang R, Schulze MB, Li T, Rifai N, Stampfer MJ, Rimm EB, Hu FB: Non-HDL cholesterol and apolipoprotein B predict cardiovascular disease events among men with type 2 diabetes. *Diabetes Care* 27:1991-1997, 2004
399. Lu W, Resnick HE, Jablonski KA, Jones KL, Jain AK, Howard WJ, Robbins DC, Howard BV: Non-HDL cholesterol as a predictor of cardiovascular disease in type 2 diabetes: the strong heart study. *Diabetes Care* 26:16-23, 2003
400. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E: High apolipoprotein B, low apolipoprotein AI, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *The Lancet* 358:2026-2033, 2001
401. Sniderman AD, Williams K, Contois JH, Monroe HM, McQueen MJ, de Graaf J, Furberg CD: A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circulation: Cardiovascular Quality and Outcomes* 4:337-345, 2011
402. Sniderman AD, St-Pierre AC, Cantin B, Dagenais GR, Després J, Lamarche B: Concordance/discordance between plasma apolipoprotein B levels and the cholesterol indexes of atherosclerotic risk. *Am J Cardiol* 91:1173-1177, 2003
403. Raitakari OT, Mäkinen V, McQueen MJ, Niemi J, Juonala M, Jauhiainen M, Salomaa V, Hannuksela ML, Savolainen MJ, Kesäniemi YA: Computationally estimated apolipoproteins B and A1 in predicting cardiovascular risk. *Atherosclerosis* 226:245-251, 2013
404. Mäkinen V, Tynkkynen T, Soininen P, Peltola T, Kangas AJ, Forsblom C, Thorn LM, Kaski K, Laatikainen R, Ala-Korpela M, Groop P: Metabolic Diversity of Progressive Kidney Disease in 325 Patients with Type 1 Diabetes (the FinnDiane Study). *Journal of Proteome Research* 11:1782-1790, 2012

405. Cleland S, Fisher B, Colhoun H, Sattar N, Petrie J: Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks? *Diabetologia* 56:1462-1470, 2013
406. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G: Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 139:802-809, 2003
407. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Hallé JP, Young J, Rashkow A, Joyce C: Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 286:421-426, 2001
408. Babazono T, Nyumura I, Toya K, Hayashi T, Ohta M, Suzuki K, Kiuchi Y, Iwamoto Y: Higher levels of urinary albumin excretion within the normal range predict faster decline in glomerular filtration rate in diabetic patients. *Diabetes Care* 32:1518-1520, 2009